PHYSIOLOGICAL ECOLOGY OF SELECTED TALITRID AMPHIPODS

THESIS

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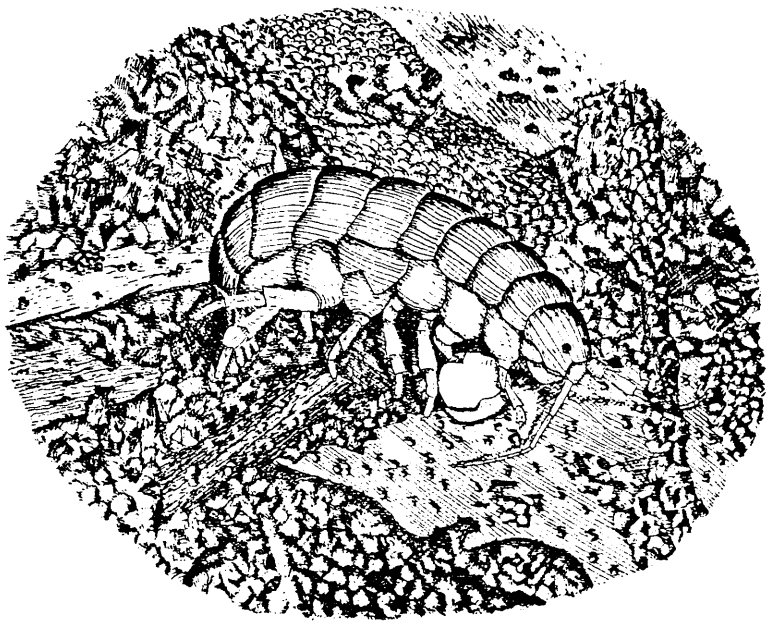
TO MY WIFE, FIONA.



And mothers loudly warned their proud pink daughters or sons to put that jellyfish down; and fathers spread newspapers over their faces; and sand-fleas hopped on the picnic lettuce; and someone had forgotten the salt.

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Acknowledgements

Firstly, I would like to thank my supervisors, Dr. Alan C. Taylor and Dr. P. Geoff Moore, for their constant encouragement, constructive criticism and good example throughout the course of this study. Thanks are also due to Professor K. Vickerman and Professor R.S. Phillips for provision of research facilities in the Zoology Department of the University of Glasgow.

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Finally, I would like to thank my wife Fiona for her constant love and support. Þæs him sie wuldor ā būtan ende.

SUMMARY

The aims of this thesis are two-fold. 1) To contribute a little to our knowledge and understanding of talitrid biology and especially the little-studied, respiratory physiology of these animals. In particular, the oxygen transporting properties of the blood of two semi-terrestrial species, have received special attention. 2) To assess the validity of Edney's (Edney, 1960) statement that "amphipods have evolved onto land by mainly behavioural rather than specifically morphological or physiological adaptations", by employing a synthesis of both the experimental work reported herein and other pertinent (though often overlooked) data drawn from the literature.

The first two chapters serve both as an introduction to the group (and the particular species studied) and as a frame of reference, allowing physiological data to be placed within a meaningful ecological context. Based both on personal observations and records together with an assessment of the literature, the distribution and natural history of these animals (particularly Orchestia gammarellus and O. mediterranea) are described. The following four chapters are concerned with original physiological investigation.

A comparison of aerial and aquatic oxygen consumption rates in four species of talitrid, taken from different habitats, revealed that a correlation exists between the ability of these animals to maintain an aerial rate of oxygen consumption following immersion and the degree of emancipation from the aquatic environment. Orchestia gammarellus and O. mediterranea were able to maintain indefinitely an aerial rate of oxygen consumption when submerged at 10^{0}C. In the case of Orchestia cavimana and Arcitalitrus dorrieni, the most terrestrial

of the amphipods examined, there was a marked suppression of oxygen consumption rate upon immersion, with A. dorrieni unable to survive prolonged submersion. A comparative study of the effects of temperature on the oxygen consumption rates for each species showed very little interspecific difference in rate increments in air and water between O. gammarellus and O. mediterranea, except at high temperatures (25^0C) at which the submerged rate of oxygen consumption of O. gammarellus was just half that of the aerial rate. In O. cavimana and A. dorrieni an increase in exposure temperature resulted in an increase in the aerial:aquatic oxygen consumption ratio. None of the species examined were able to sustain aerial respiration at temperatures in excess of 30^0C and aquatic respiration at temperatures in excess of 25^0C. Gill excision in O. gammarellus did not significantly alter the oxygen consumption rate of this animal except at high (>20^0C) exposure temperatures. These results were discussed in relation to the invasion of land in this group.

The total gill area and gill distribution of seven talitrid species taken from a variety of habitats has been studied. In the sandhoppers, Talitrus and Talorchestia, and in the landhopper, Arcitalitrus dorrieni, the greatest gill area is invested in the posterior gills (G 6) whereas in the beachflea, Orchestia spp. G2 has the greatest area. Gills 3, 4 and 5 are characteristically small and G 7 is consistently absent in all the species studied. Gill structure and deployment patterns are, in general, quite conservative within the Talitridae. The gills of A. dorrieni are, however, complex in shape and are more convoluted than in the other species studied.

Gill area/ body dry weight relationships were established for each species and were compared with previously published data. The Talitridae show a reduction in total gill area compared with most aquatic amphipods. It was observed, however, that the slopes of the

calculated regression lines were significantly different between the sandhopper and beachflea genera studied. The reduction in gill area within the Talitridae is discussed in relation to their colonization of land.

A comparative study of the oxygen transporting properties of the blood of two supralittoral talitrids was attempted. This is the first account of the oxygen transporting properties of amphipod haemolymph. The concentrations of the major ions in the blood were similar in the two species although in O. mediterranea the concentrations of the Na^+ and Cl^- ions were lower than in O. gammarellus. The oxygen carrying capacity of the haemocyanin was quite low (0.7 ml.100 ml^{-1}) and did not differ significantly between the two species. The blood of both O. gammarellus and O. mediterranea had a moderately high oxygen affinity which remained almost independent of temperature in the range 10 - 18^0C. The Bohr factor ($\Delta \log P_{50} / \Delta pH$) was almost identical in the two species (mean = -0.84). The cooperativity of the haemocyanin was also similar in both O. gammarellus and O. mediterranea (n_{50} = 3) and was largely independent of pH within the pH range 7.2 - 7.8.

It was found, however, that the O. gammarellus collected from the upper limits of their distribution on the shore (designated AHW animals) had a blood ion concentration that was very different from animals taken from the middle of their distribution (designated BHW animals). The concentrations of Na^+, Cl^-, and Ca^{2+} ions in particular were very much lower in the blood of animals from high on the shore and the pH of the blood was significantly lower (7.63 compared with 7.83). As a result, the in vivo oxygen affinity of the blood of AHW animals was much lower than that of BHW animals. When measured at constant pH, the oxygen affinity of the blood of AHW animals was still lower than that of BHW animals. Experiments in which

blood from the two groups of animals was dialysed against different physiological salines, showed that this could be attributed to the lower Ca^{2+} ion concentration in the blood of AHW animals. The oxygen transporting properties of the blood are discussed in relation to the ecology of these species.

The results of a preliminary study of the effects of long term hypo- and hyper-saline exposure on O. gammarellus, at the level of the tissues, are given to supplement the account of the oxygen transporting properties of the blood. It was found that O. gammarellus was able to regulate the sodium ion concentration of the blood under hypo-osmotic conditions and that there were accompanying changes in the animals' total free amino acid and total blood protein concentrations. From both laboratory and field data, it was noted that a decrease in the blood ionic concentration (AHW animals) is very often accompanied by an increase in the overall protein concentration of the blood. The results are discussed in relation to the role of haemocyanin as a source of free amino acids to maintain the colloidal osmotic pressure of the blood when the animal is under hypo-osmotic stress.

The last chapter is given over to a general discussion of the results and relevant literature, compounded with a critical assessment of Edney's statement as regards land colonisation by the Talitridae. This assessment is carried out within the systematic-ecological framework recently provided by Bousfield (1984).

1.1 INTRODUCTION

The transition from the marine to the terrestrial environment is a major one. The change in the surrounding medium demands a complete change in the primary mode of progression and need for modifications in the basic processes of life; reproduction, respiration and excretion. Greater extremes of temperature fluctuation occur on land, and with the constant threat of desiccation, terrestrial animals may be expected to display varying degrees of physiological adaptation and tolerance of aerial conditions.

There are a number of comprehensive general accounts of the transition from water to land and many more which highlight particular taxonomic groups (See Little, 1983 for review). Only three groups of crustaceans, the Isopoda, the Decapoda and the Amphipoda, have achieved some measure of success on land (Powers and Bliss, 1983). Much of the information accumulated up until 1968, together with other contemporary work, was presented at a symposium of the American Society of Zoologists organised by Bliss and Mantel (1968). Two recent reviews (see below) of terrestrial adaptations in the Crustacea draw heavily from this work. There are a few publications (see Chapter 7) which have been concerned specifically with the problem of land colonisation in the Talitridae, the only family of amphipods which has truly terrestrial representatives (Hurley,1968).

In Milne-Edwards' (1840) treatise, "Histoire Naturelle des Crustacés", only thirteen semi-terrestrial talitrid species were described but there was no reference to amphipods in relation to the land habitat; he merely referred to talitrids and orchestids living by the sea. The first reference to fully terrestrial amphipods was published by Dana in 1852 although he had made the discovery 12 years previously. He recorded the presence of Orchestia sylvicola from 20 miles inland, in humid soil in New Zealand and Orchestia tahitensis

from beneath dead leaves at an altitude of 500m in Tahiti. In his "Catalogue of the specimens of Amphipodous Crustacea in the collection of the British museum", Bate, (1862) listed another 26 semi-terrestrial species but added only one more euterrestrial species to those discovered by Dana. This situation was altered when Heller (1865), in a note on fresh-water amphipods, included a description of Orchestia cavimana which he found in large numbers in humid environments at a height of 1500m on Mount Olympus.

In 1888, a number of accounts were published concerning the existence and adaptations of talitrid amphipods in relation to the land habitat. These included three separate accounts resulting from visits to the Azores by the Frenchmen; Barrois, De Geurne and Chevreux. Unfortunately these papers have been overlooked by subsequent reviewers of the subject. Chevreux (1888) noted that, "A cent mètres de la mer, et à une altitude de 15 mètres, l'Orchestia littorea abondait sous les pierres humides qui garnissaient les abordes d'une petite fontaine d'eau douce." Both he and De Geurne (1888) recognised the existence of various degrees of terrestrial adaptation in the Talitridae and discussed the role of humidity in delineating their habitat. Barrois (1888) added nothing new to the observations of his countrymen, concentrating primarily on emphasising the existence of fully terrestrial amphipods, a fact that unfortunately has had to be reiterated on many subsequent occasions (see Hurley, 1968). In the same year in Scotland, David Robertson of Millport, in his series of studies on the Amphipoda and Isopoda of the Clyde (Robertson, 1886, 1888), commented on the adaptations to living above the high water mark he had observed in Talitrus saltator and Orchestia gammarellus. Despite the crudity of his methods, he would appear to have been one of the first investigators to approach this

subject experimentally.

The year 1880 saw the description of Talitrus sylvaticus from Australia by Haswell (1880), the first of many accounts concerning the large fully terrestrial Indo-Pacific fauna (see below). At this present time more than 200 talitrid species have been described with possibly more than 1000 fully terrestrial species (ie. excluding supralittoral forms) awaiting description (Bousfield, 1984).

There are some recent papers that deal specifically with the transition from water to land in the talitrid amphipods (Hurley, 1959, 1968; Friend and Richardson, 1986; see also the brief but interesting note in Barnard, 1972) and also two recent reviews, each containing a section on the terrestrial adaptations of this group (Little, 1983; Powers and Bliss, 1983). The papers of Hurley (1959, 1968) and of Friend and Richardson (1986) dealt primarily with the fully-terrestrial "landhopper" species of the southern hemisphere with particular reference to changes in their morphology and distribution and the routes that these animals have taken onto land. They also briefly considered some physiological data from both published and unpublished studies on semi- and fully terrestrial amphipods, but in each case their assimilation of such experimental work into the main thesis or their respective papers is limited. This may be due to the paucity and inappropriate nature of the experimental evidence available and may also reflect their own interests.

The reviews of Little (1983) and Powers and Bliss (1983), however, were more cosmopolitan, if not comprehensive, in their scope and included discussion of the adaptations of both supralittoral and terrestrial species. Together they illustrate the lack of detailed knowledge of talitrid biology and physiology in particular. Edney (1960), in an article in which he reviewed the physiological adaptations underpinning "terrestrialness" in the Crustacea, suggested

that, "the Amphipoda have adapted to land more by behavioural mechanisms than specific physiological or morphological means." This statement was reiterated in one or Hurley's papers (Hurley, 1968) as well as in both of the reviews mentioned above. Edney's article, however, was more concerned with the terrestrial isopods and only five papers dealing with aspects of amphipod biology were quoted. It would appear that much of the reasoning applied to talitrid physiology and its relationship to the land habitat (with some notable exceptions: forexamplesee Williamson, 1951a) has been extrapolated from other groups (mainly the isopods and to a lesser extent the decapods) in the absence of specific experimental data. Within the last few years, however, a renewed interest has been shown in original investigation of the physiology of these amphipods as in relation to their terrestrial habits (Marsden, 1980, 1984, 1985; Lazo-Wasem, 1984; Moore and Taylor, 1984; Moore and Francis, 1985a,b, 1986a). These studies complement and add to the earlier physiological studies of such pioneer workers as Backlund, Pallaudit and Williamson (see later chapters for full details).

1.2 TALITRID SYSTEMATICS

It remains true, however, that most studies on talitrid amphipods have been of a systematic nature, occasionally including notes and observations on the biology of these animals. The British fauna has been adequately described (Bate and Westwood, 1863; Stebbing, 1891; Reid, 1947; Lincoln, 1979) as has that of the North Atlantic and Mediterranean regions (Grube, 1866; Boeck, 1872; Chevreux, 1893, 1895; Sars, 1890 ; Stebbing, 1906a; Schellenberg, 1942; Dahl, 1943 ,1946, 1964 ; Chevreux and Faye, 1925; Vecchi, 1929; Dons, 1935; Den Hartog, 1963; Amanieu and Salvat, 1963a,b, 1965; Karlbrink, 1969; Karaman, 1970; Ingolfsson, 1974, 1977 ; Beemster and Dezwart, 1977; Calvario

and Marques, 1983; Marfin, 1983). There are, however, new species still being described from these areas (Amanieu and Salvat, 1963a,b). With the exception of species introduced into ornamental gardens and greenhouses (Chevreux, 1896; Bonnier, 1898; Menzel, 1911; Calman, 1912; Hunt, 1925; Heimlich, 1930; Tattersall, 1931; Medcof, 1939; Palmen, 1949; Vader, 1972; Bierbaum, 1980; Richardson, 1980; Lazo-Wasem, 1983; Moore and Spicer, 1986), the talitrid fauna of the Northern hemisphere is composed of semi-terrestrial "beachfleas" and "sandhoppers". This is not totally true, however, of the Azores or the Canary islands (De Geurne, 1889; Dahl, 1950, 1967; Andersson, 1963; Alfonso, 1977; Cejas et al., 1983). The fauna of the coasts of the U.S.S.R. and her satellites have been described, primarily in the work by Bulycheva (1957) (see also Drzycimski and Nawodzinska, 1965).

The systematics of the North-West Atlantic and the Indo-Pacific region too have been the subject of extensive, if erratic, examination (Holmes, 1904; Stebbing, 1903, 1908b; Ueno, 1929, 1935; Shoemaker, 1930, 1932, 1942; Stephenson, 1935a,b, 1939; Iwasa, 1939; Thorsteinson, 1942; Bowers, 1963, 1975; Bousfield, 1964, 1971, 1976; Morino, 1972). In particular the western and eastern coasts of the United States have latterly received much attention from Dr. E.L. Bousfield and his co-workers (Bousfield, 1957, 1958, 1961, 1973, 1975, 1982, 1984; Bousfield and Carlton, 1967; Bousfield and Klawe, 1963; Bousfield and Heard, 1986). Some descriptions of the South American fauna have also been published (Schellenberg, 1938a; Ruffo, 1950; Castro, 1972, 1979; Soares, 1979; Varela, 1983).

Supralittoral talitrids are quite distinct from the euterrestrial "landhoppers" which, in the main, inhabit the forest floors of the Indo-Pacific region, and are restricted to the southern hemisphere (Hurley, 1959, 1968). In the last hundred years much effort has gone

into describing fully terrestrial species found in Australia and New Zealand (Haswell, 1880, 1882, 1885; Thomson, 1879, 1880, 1893, 1899; Walker, 1908; Chilton, 1909, 1916a,b, 1917, 1919, 1925; Hurley, 1955, 1956, 1957; Macintyre, 1963; Duncan, 1968; Fearn-Wannan, 1968; Friend, 1979, 1980, 1982; Marsden and Fenwick, 1984), in Africa (Chevreux, 1902a, 1908; Methuen, 1913; Barnard, 1915, 1940, 1958; Stebbing, 1917, 1922; Schellenberg, 1938b; Ruffo, 1958), in India (Tattersall, 1914; Carl, 1934; Burt, 1934; Barnard, 1935, 1936; De Sylva, 1959), in Malaysia (Stebbing, 1886; Baker, 1915; Tattersall, 1929; Barnard, 1936, 1960) and the Pacific Islands (Stebbing, 1900; Bousfield and Howarth, 1977; Bowman, 1977).

It has long been recognised, however, that the generic criteria applied to these animals was inadequate (see Hurley, 1956,1975 for example). Separation of the species into their respective genera eg. Talitrus, Orchestia, relied solely upon the form of the gnathopods (Nicolet in Stebbing, 1888), dividing the species into sexually dimorphic and sexually similar genera. The internal divisions of the former category have also been the subject of much debate as they do not clearly differentiate euterrestrial species and morphologically similar forms from the supralittoral zone. Previously, those species had been lumped indiscriminately together with such genera as Orchestia and Talitrus. Stebbing (1906) arbitrarily defined "Orchestia" and "Parorchestia" to differentiate semi- and fully terrestrial forms. Bonnier (1898) too set up a genus, Talitroides in an attempt to encompass species that were characterised by the absence of distinct sexual dimorphism. Both of these schemes have proved unsatisfactory. Some recent studies (Friend, 1979, 1982; Bousfield, 1982, 1984), however, have attempted to classify these species using more precisely defined characters of the mouthparts, peraeopods and other appendages as well as the gnathopods. This has resulted in the

recognition of four talitrid ecomorphotypes (Bousfield, 1984) which encompass not only the euterrestrial "landhoppers" but also the remaining littoral and supralittoral genera.

Group I is composed of palustral talitrids; these semi-aquatic amphipods are estuarine or freshwater animals being found in tropical and antipodean continental areas. Five new genera have thus replaced Stebbing's (1900) Parorchestia.

Group II is composed of more advanced forms than the palustral amphipods of group I, and contains both semi- and fully terrestrial members. They are called beachfleas, although as noted above, they are not restricted to the supralittoral zone. This non-substrate modifying group (MacIntyre, 1963) contains the common shorenopper of British shores, Orchestia gammarellus as well as a number of new genera. Animals belonging to this group are found on both tropical and boreal coastlines.

Group III, the sandhoppers, is composed of semi-terrestrial, fossorial amphipods such as Talitrus saltator. They are found predominantly on sandy beaches of temperate and tropical marine shores around the high water mark.

Finally, Group IV contains the landhoppers. They are specialised euterrestrial animals found not on the shore but mainly in continental and island rainforests of the tropical Indo-Pacific region. This group is further sub-divided as outlined in Chapter 7. Only one landhopper species occurs free-living in the British Isles, Arcitalitrus dorrieni, which was accidentally introduced into this country about 100 years ago from Australia (Moore and Spicer, 1986).

1.3 AIM OF THESIS

In the light of the systematic scheme constructed by Bousfield (1984), there has been a renewed impetus for comparative physiological studies of the different eco-morphological groupings. Recent years have also witnessed a trend towards the experimental study of adaptations shown by terrestrial animals (see Edney, 1960, 1977) rather than to theorise about the routes and causes of their movement from water to land. While the study of comparative talitrid physiology reveals present ecological adaptation, this needs to be set in the context of past evolutionary history of the group to produce a more comprehensive and realistic organismic biology.

Towards this end, this thesis examines some aspects of the respiratory adaptations of two British talitrid species, Orchestia gammarellus (Pallas) and O. mediterranea (Costa). The results are placed within the context of the natural history of these species. During the course of the study, some other talitrid species became available, facilitating interesting interspecific comparisons within the Talitridae. Finally, present results and data from the literature are assessed within the eco-morphological evolutionary framework proposed by Bousfield (1984).

CHAPTER 2

NATURAL HISTORY

2.1 INTRODUCTION

There is, at present, no comprehensive account of the natural history of any single species or group of talitrid amphipods. There are a few early monographs on individual beachfleas (eg. Smallwood, 1903, 1905) but the modern depreciation of broad natural history has left perhaps some of the most abundant and conspicuous animals of the seashore, almost unknown to scientist and layman alike. This may account for apparent misidentifications even in recent scientific literature (eg. Elliot et al., 1983; see Bagenal, 1957 on Gauld et al., 1953. Both identify as Talitrus saltator, animals which are more likely to be O. gammarellus). Much of this thesis deals with the physiology of these animals, so it is appropriate at the outset to collate what is already known of their biology. This review has been supplemented by original observations and distribution records made over the period August, 1982 to January, 1986.

The chapter is divided into five sections. The first comprises an account of the natural history of Orchestia gammarellus (Pallas) (Semi-terrestrial Group II, Bousfield, 1984) and O. mediterranea (Costa) (Eulittoral or semi-terrestrial, Group II, Bousfield, 1984), since much of the experimental work reported later was concerned with these two species. It should be noted, however, that much of this first section is also relevant to the additional species under consideration in the following shorter sections.

The next four sections include distribution data for all of the species studied in Chapters 3 - 7. Natural history observations are also included for each of the remaining species under consideration, viz. Orchestia cavimana (Heller) (Freshwater: semi- to fully terrestrial: Group II, Bousfield, 1984), Talitrus saltator (Montagu), Talorchestia deshayesii (Audouin) and Talorchestia sp.A (all semi-terrestrial; Group III, Bousfield, 1984) and Arcitalitrus dorrieni

(Hunt, 1925) (Euterrestrial: Group IV, Bousfield, 1984).

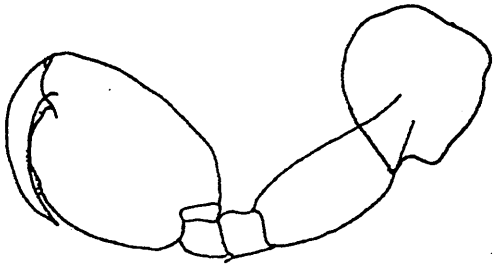
2.2 ORCHESTIA GAMMARELLUS AND O.MEDITERRANEA

2.2.1 General morphology

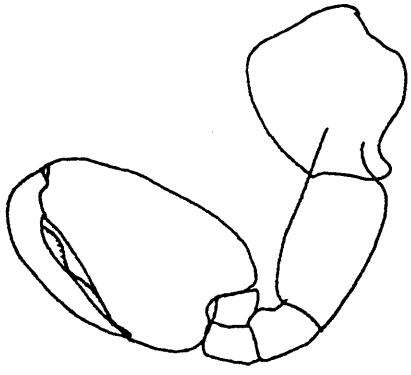
Both Orchestia gammarellus (Pallas) and O. mediterranea (Costa) live in close proximity to the sea and are quite distinct from the "landhoppers" which inhabit the forest floor of the Indo-Pacific region (Hurley, 1959, 1968). The morphological differences between these two species and the systematic separation of all the British Talitridae (Anon, 1979) can be found in Lincoln (1979). Briefly, the main diagnostic features are as follows. In the male, the species can be easily distinguished one from another by the differing shapes of the second gnathopoda. The palm of the propodus of O. gammarellus is more than half the length of the posterior margin and is regularly convex (Fig. 2.1a). In O. mediterranea the palm is less than half the length of the posterior margin, being sinuous with a median hump (Fig 2.1b) (Reid, 1947; Lincoln, 1979). The females of both species can be differentiated by the pleopod ramus:peduncle ratio. In O. gammarellus the rami are equal to the length of the peduncle whereas in O. mediterranea they are only about half the length (Williamson, 1949b). The maximum body length in both species is approximately 20mm (Barrett and Yonge, 1958; Wildish, 1972). Females of both species are normally slightly smaller than males (Bales, 1961). The colour of these animals is predominantly brown but there is a great deal of variation even between animals found beneath the same stone (Reid, 1947). In life, the integument of O. gammarellus bears many small brown markings on the dorsal surface, but they disappear on preservation in either 70% alcohol or 10% formaldehyde (formalin) solution; animals turn a very

FIG. 2.1 The second gnathopod of male Orchestia gammarellus (A) and Orchestia mediterranea (B).

A



B



bright carrot-red colour when placed in the latter, while in the former it is not uncommon for the exoskeleton to turn a uniform white. Large males of O. mediterranea from the West coast of Scotland are very often characterised by the dactylus of the second gnathopod being a bright red colour (see also Bate, 1862 and Reid, 1947).

2.2.2. Habitat and distribution

Orchestia spp. are commonly known as "shorehoppers", as distinct from the "sandhoppers" such as Talitrus saltator and Talorchestia deshayesii; the different names reflect a difference in their habitats (Bate and Westwood, 1863; Robertson, 1886). In places where the various habitat types intergrade, "sandhopper" and "shorehopper" species can be found in close proximity (Fig. 2.2). Orchestia gammarellus has a very wide habitat range from intertidal marine and estuarine areas (Fig. 2.3) to damp semi-terrestrial habitats well away from water (Dahl, 1946; Scott, 1956; Den Hartog, 1963; Ingolfsson, 1977; Nelson, 1980; Moore, 1984; see also Fig. 2.4). In fact this species has been found 120m above sea level on the Island of St. Kilda (Bagenal, 1957) and just over 50m above sea level on the Island of Skye (J.I.S. pers.obs.). The species is most frequently found, however, beneath decaying wrack and debris around the high water mark on predominantly rocky or shingle shores (Fig. 2.5) (Robertson, 1868; Sars, 1890; Bousfield, 1958; Karlbrink, 1969; Perkins, 1974; and plays an important role in the biodegradation of stranded algal material (Backlund, 1945).

In laboratory studies (Perkins, 1974), it was found that although Orchestia (probably O. gammarellus but Perkins is not specific) ingested litter at the surface of the shingle, it habitually lived at a level within the shingle just above that of the water table. Backlund (1945), noted however, that in large wrack beds cast up by

FIG. 2.2 Ballochmartin Bay, Great Cumbrae Island (5.6.85)

Four species of talitrid can be found in this bay, their different spatial distributions illustrating the difference between "sandhoppers" and "beachfleas". Beneath the wrack featured in the foreground and on the rocky promontory in the top right hand corner (A), O. gammarellus and O.mediterranea are very common, the former extending its range up onto the grass verge above the beach proper. Orchestia mediterranea is found at the level of the lowest wrack string (B). The "sandhoppers", as the name suggests, are restricted to the sandy area enclosed by the rocks (C). Talorchestia deshayesii was generally found at a higher level than Talitrus saltator on this beach. Where both rocky and sandy shore intergrade as in the bottom right hand corner of the picture, it was not uncommon for both sand and beach hoppers to co-occur.

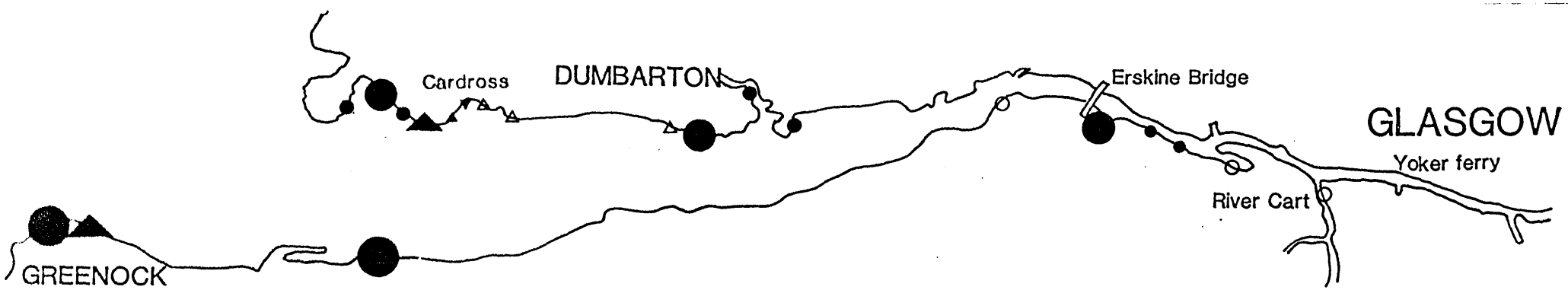

A
B
C

FIG. 2.3 The distribution of Orchestia gammarellus and O. mediterranea in the Clyde estuary.

The distribution of O. gammarellus and O. mediterranea in the Clyde estuary was monitored using field sampling methods similar to those employed by Wildish (1970b). This allowed a valid comparison of Wildish's study in the Medway estuary and this present preliminary study. Animals were picked up between thumb and forefinger after turning over stones and debris between EHWS and MHWN. The relative density was expressed as the number collected per 5 minutes collecting time per square metre. Animals were returned live to the laboratory, identified and examined under a binocular microscope (X10).

Orchestia gammarellus extends further up river than O. mediterranea. The latter species reached its estuarine limit at Cardross on the north side of the estuary. The barrier was well defined (7.1.85), with the last individuals of O. mediterranea occurring 200m west of Cardross railway station. The tergite patterns of this species at the limits of their distribution were identical to individuals taken from the Isle of Cumbrae the previous month. It is unlikely that this species exists as two morphs at this location as has been recorded in the Medway estuary (Wildish,1970b). Estuarine penetration is also less marked in the Clyde population compared to that examined by Wildish (1970b) from the South-East of England.

The upper limit for O. gammarellus varied over the two years the populations were examined. In spring 1984, O. gammarellus extended up river as far as the Erskine Buoy (NS 64 713468) but a



March, 1985

Relative density

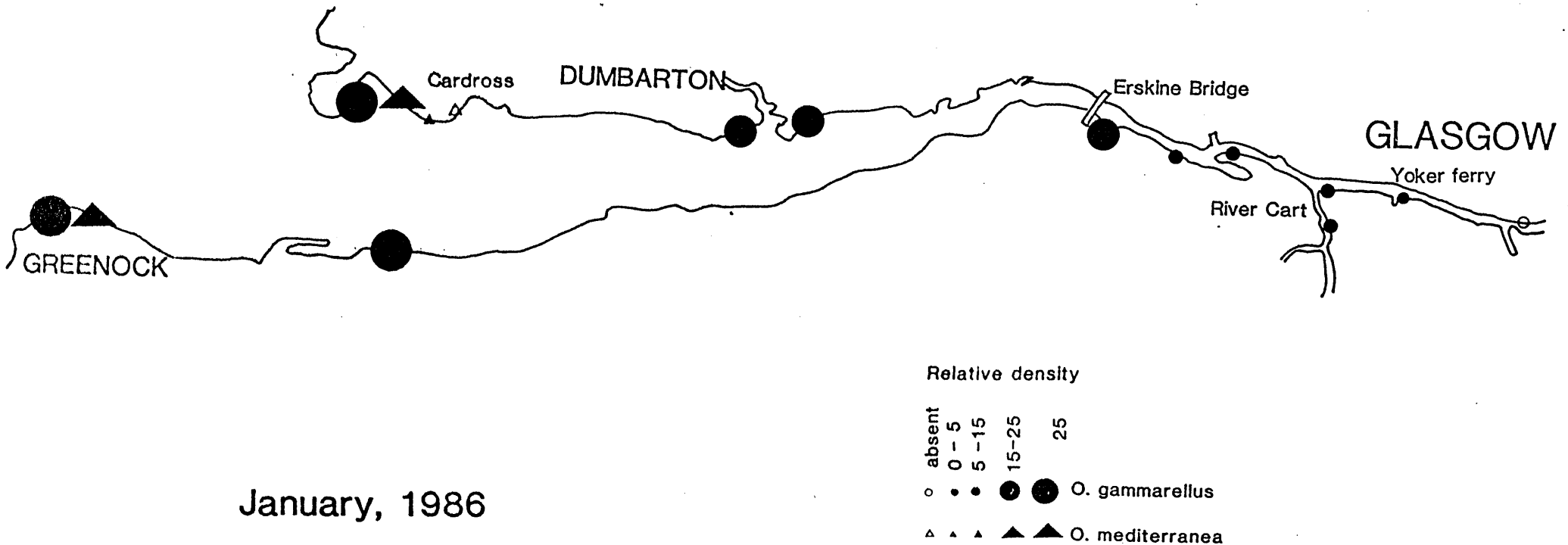
absent
0 – 5
5 –15
15–25
25

O. gammarellus

O. mediterranea

FIG. 2.3 continued

few hundred metres further up at Rashlee light, amphipods were consistently absent at several different sites (A). The following spring however,it was noted that O. gammarellus was present both at the mouth of the River Cart and as far up-river as the Renfrew Ferry slipway, where individuals were found beneath characteristically fresh-water debris (B). At high tide, the salinity of the water was 9‰, which is considerably higher than for the limit found by Wildish (1970b) in the Medway Estuary. This may be due to the lack of appropriate habitat types in the dockland area and further upstream, or some inherent difference in water quality. The reason for the shift in the limit of O. gammarellus between 1984 and 1985 is as yet undetermined though it is likely tnat it may related to differences in the annual temperature/ salinity regime.



January, 1986

FIG. 2.4 On December 8th, 1984, much debris and weed was cast up onto the land and even onto the perimeter road on the Isle of Cumbrae, during a storm. On close examination of this debris, it was found that many amphipods (predominantly O. gammarellus) were present. Thereafter, one particular spot on Farland Bight was visited at monthly intervals, to check for the presence of amphipods. In the figure opposite, general flotsam and jetsam can be seen at the bottom right hand side of the picture, many metres from the top of the beach. Although this picture was taken in June, 1985, O. gammarellus was still present at this location, although not in such great abundance as noted six months previously. In the majority of cases, it is unlikely that active dispersal is responsible for the inland records of O .gammarellus in this country.

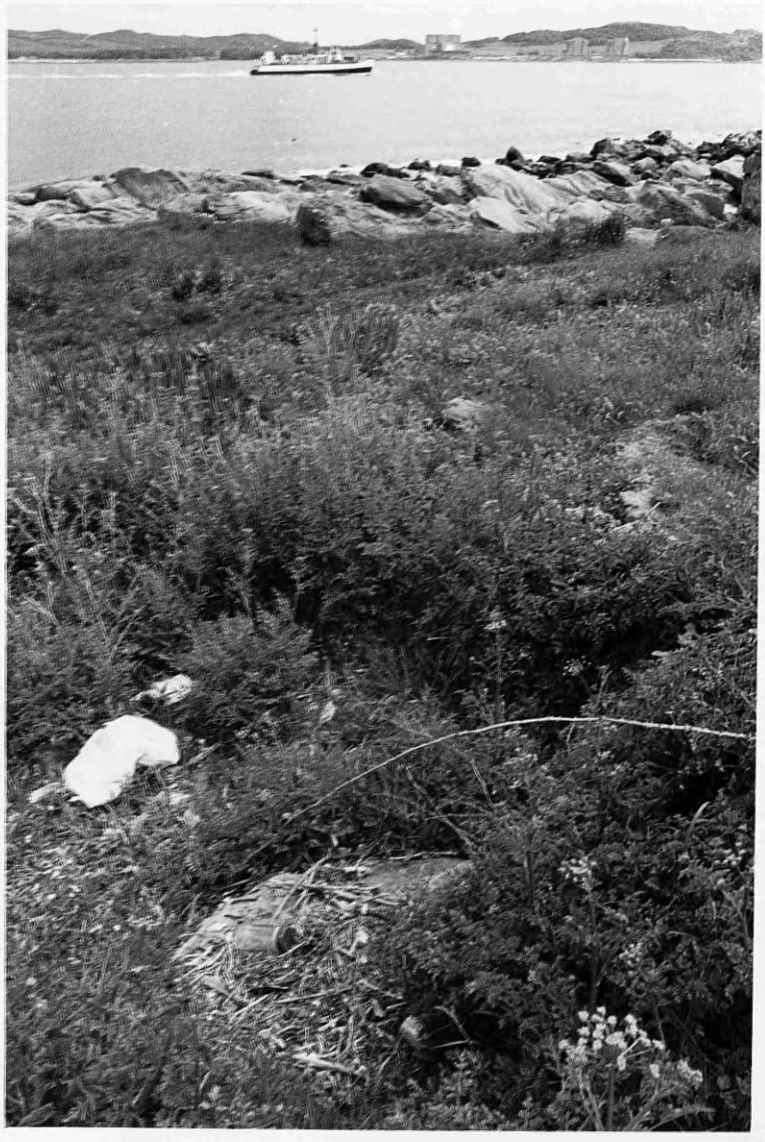


FIG. 2.5 Rocky shore habitat of Orchestia gammarellus.

In Fig. 2.5a is a photograph of the rocky shore at Farland Bight Great Cumbrae Island. Orchestia gammarellus was present in abundance, both within and beneath the large wrack accumulation (centre) and in the wrack string (upper left) at the top of the beach. O. mediterranea was present beneath stones just below the level of the large wrack bed. Figure 2.5b) is of the rocky shore, south of Fintray Bay, Great Cumbrae Island. Orchestia gammarellus was present both within the wrack accumulations (bottom, left) and beneath the sandstone slabs lying on the sandstone pavement feature in the background. The A.H.W. animals (see Chapter 5) were collected from beneath rocks at the grass margin of the beach.

a

b

the tide, most O. gammarellus were concentrated in a " border" zone 2cm deep: the deeper layers were full of decaying weed and Orchestia excrement while the surface layers were composed of unaltered algae. Perkins (1974) found that while defaecation occurred upon the litter, most faecal pellets accumulated at the bottom of the experimental jar. His examination of a shingle bank at Coldbackie in Sutherland, confirmed this. He found an abundance of faecal pellets at a depth greater than 0.3m within the shingle. It is probably true to say that both superficial wrack and underlying shingle are inhabited by Orchestia but the amphipods' vertical zonation at any given time may depend upon a combination of environmental factors. Backlund (1945) found that O. gammarellus quite literally ate its way upward in a wrack bed as far as the wrack remained cool and humid enough, while on hot, sunny days they retreated to the deeper layers.

Orchestia gammarellus extends along the European coasts from western Norway and Denmark (Boeck, 1872; Sars, 1890; Dahl, 1946), Sweden and the Baltic (Karlbrink, 1969), the Deltaic region of Holland (Den Hartog, 1963), the coasts of the Britisn Isles (Lincoln, 1979; see also Fig. 2.6a) and France (Chevreux and Fage, 1925) down into the Mediterranean and Black Seas (Caspers, 1951). It is also found on the Canary Islands (Barrois, 1887; Dahl, 1947), the Atlantic coast of Canada (Bousfield, 1958) and the extreme west coasts of Iceland (Ingolfsson, 1974). Ingolfsson (1977) notes that O. gammarellus on Iceland is limited to locations where the average air and sea temperatures are both high. He also notes that while marine species "drop out" in the same order on both sides of the Atlantic (as well as around Iceland) as one moves into colder temperature regimes two exceptions stand out. Both O. gammarellus and Hyale nilssoni extend considerably further into a cold temperature regime on the North American coast than on European coasts (including Iceland) relative to

FIG. 2.6 Distribution of Talitridae in Scotland

The four native species of Scotland (O. gammarellus [A], O. mediterranea [B], Talitrus saltator [C] and Talorchestia deshayesii [D]) are found wherever their respective substatum preferenda are fulfilled. The West coast of Scotland supports large populations of Orchestia spp., mainly due to the prominence of rocky habitats. However, these animals were found at various levels of abundance all round the coast. There were even some localised populations existing beneath debris, lying on a small area of rocks on a predominantly sandy beach (Lochinver, June, 1985). The distribution of Talitrus and Talorchestia can largely be correlated with the distribution of clean sandy beaches. Consequently, the sandy beaches, particularly from Loch Torridon to Cape Wrath and from Nairn, right down the East coast past Aberdeen, support a very large fauna of sandhoppers. As is the case with Orchestia spp., many localised populations may exist on just a few metres of sandy beach (eg. Ballochmartin Bay, Isle of Cumbrae. see Fig. 2.2).


A
B
C
D

other species. He regards this as evidence that their distributional limits are controlled in a fashion different to that of other more marine species and that these supralittoral species may be more affected by air temperature than are other intertidal species (see also Moore, 1977 on H. nilssoni).

Orchestia mediterranea is a predominantly warm water species, being found in the Mediterranean (Chevreux, 1893; Ruffo, 1937, 1938; Louis, 1977a) and having its northern limit in the North of Scotland (Fig. 2.6) and the Deltaic region of Holland (Den Hartog, 1963; Lincoln, 1979). It is absent from Iceland and the Scandinavian countries. Unlike O. gammarellus, O. mediterranea is not usually found above the high water mark (Reid, 1947; Moore, 1984) although occasional specimens have been taken from above this level (Bate and Westwood, 1863). In general, O. mediterranea is found to occupy a fairly narrow zone on the shore (living below the zone of O. gammarellus) where it is inundated at each high tide. This involves appropriate movements up- or down- shore at spring or neap tides (Chevreux and Fage, 1925; Den Hartog, 1963; Wildish, 1982a). The amphipods live under rocks and stones at the same shore level as the fucoid, Pelvetia canaliculata. Wildish (1982a) states that this is a eulittoral animal since it is dependent on the daily tidal level as well as on the wrack on which it feeds.

Orchestia mediterranea is a polymorphic species. Near to its limit of penetration into estuaries, the typical morph O. mediterranea mediterranea is replaced by morph O. m. aesturensis which differs from morph mediterranea in dorsal pigment patterns, in the incidence of intersexes and in having a high proportion of reproductive females (Wildish, 1970b,c).

2.2.3 Ecology

When O. mediterranea co-occurs with O. gammarellus, the former is usually (though not invariably) in the minority (Den Hartog, 1963). There are reports that at some locations in the British Isles, populations of both species occur at the same shore level (Goodhart, 1941; Wildish, 1970b). This, however, is not the case in the Firth of Clyde where there is a clear segregation of O. mediterranea from O. gammarellus (although some individuals from the lower fringe of the O. gammarellus zone do mix with O. mediterranea, see Moore (1984). Perhaps as O. mediterranea reaches its northern geographical limit, its ability to extend further up the shore is limited by climatic factors such as air temperature. This would reduce its competitive ability against O. gammarellus. It is known that O. gammarellus undergoes a vertical migration on the shore corresponding with seasonal changes in habitat; the animals move further up the shore in winter and retreat again during the summer (Karlbrink, 1969; D.J.Agnew, pers.comm.). The effect of this migration on the distribution of O. mediterranea is at present not known.

The ecology of British Orchestia spp. has not been studied in any great detail with the exception of Wildish's (1970b) work on the Medway estuary. Although he presented no quantitative data, Den Hartog (1963) examined the ecological relationships of O. gammarellus and O. mediterranea particularly with regard to their differing heights on the shore. He thought that interspecific competition potentially existed between these species, but that its effect was largely attenuated by their differing ecological requirements. In the Deltaic region of Holland, O. mediterranea extended downshore further than O. gammarellus; the lower part of the range of O. mediterranea may be a refuge, where it can retreat from competition. In the absence of a competitor, in Holland, O. mediterranea still occupies a band further

down the shore in areas never occupied by O. gammarellus. The reverse was not the case in the Firth of Clyde where, if O. mediterranea was absent, its place was taken by O. gammarellus. Den Hartog (1963), however, considers that, when ecological circumstances are favourable, O. mediterranea may intrude into the territory of O. gammarellus to the extent that, "when the degree of moisture is optimal for O. mediterranea, this species may be a fervent competitor driving out O. gammarellus."

It is known that competition and predation are important in determining the zonal distribution of some other amphipod species (Vince et al., 1976; Van Dolah, 1978; Fenchel and Kolding, 1979). Vince et al.(1976) suggested that predation by Fundulus heteroclitus (the killifish), was a dominant factor in restricting the distribution of the salt-marsh amphipod Orchestia grillus to high intertidal habitats. Kneib (1982), however, employing transplantation experiments, found that the intertidal distribution of O. grillus and the species occurring immediately below (Gammarus palustris) were determined primarily by preferences for specific physical conditions. In general, the effect of environmental factors on talitrid survival and distribution is poorly documented and detailed studies are urgently required. The moist habitat and nocturnal activity of semi-terrestrial amphipods (Bowers, 1964; Nelson, 1980) are generally regarded as adaptations arising from their inability to control water-loss to the atmosphere (Dorsman, 1935; Williamson, 1951a; Hurley, 1968; Lazo-Wasem, 1984). It is known that O. gammarellus is extremely sensitive to desiccation (Backlund, 1945; Williamson, 1951a) but paradoxically less so, it would appear than the euterrestrial Arcitalitrus sylvaticus (Lazo-Wasem, 1984). (This will be discussed in more detail in the last chapter.) Unfortunately there are no data

available for O. mediterranea and so a comparative study of desiccation resistance in relation to relative position on the shore of the two species is not possible at present. Dahl (1946) assumed that differences found between humidity tolerance of O. gammarellus and Platorchestia (=Orchestia) platensis (Backlund, 1945) were partially responsible for allowing the former to exist in areas (ie damp crevices with no wrack cover) where the latter did not occur. Platzman (1960) found a slight difference in humidity tolerance between Talorchestia megalophthalma and Orchestia agilis, but not enough, he thought, to restrict them to different intertidal habitats. It may be in Platzman's case, as indeed with the two Orchestia spp. under present consideration, that behavioural and distributional differences between two such closely related species will be found to be more important than humidity tolerance per se (or any other physical parameter) in differentiating their respective niches.

Wherever they are found, both O. gammarellus and O. mediterranea may occur at very high densities (Fig. 2.7). Venables (1981c) found the supralittoral Talorchestia margarita at densities of 5,592- 39,047 m^2, which is exceptional among amphipods (Gammarus oceanicus, <10,000 m^2 but usually 1,000 m^2 or less (Steele, 1976); Pontogammarus maeoticus, 4,821 m^2 (Kasymov, 1976). Other supralittoral talitrids, eg. Orchestia tuberculata (1,000 m^2) (Duarte, 1974) or Talorchestia quoyana (256 m^2) (Fincham, 1977) occur at much lower densities. During the present study, O. gammarellus was found at densities exceeding 60,000 m^2 in some areas of South Fintray Bay, Great Cumbrae Island. Such figures however, are greatly dependent on the amount of cast weed that has accumulated on a particular beach and can vary dramatically within a comparatively small area.

Aggregation behaviour in the Talitridae is poorly studied compared with woodlice. Personal observations indicate that Orchestia

FIG. 2.7 Concentration of Orchestia gammarellus disturbed on removal of wrack covering (X3).


10

spp. are most active when contact with surrounding surfaces is minimal. Once the trunk of the body, ie. apart from the peraeopods, comes into contact with a surface (even if that surface is another animal), the animal becomes less active finally becoming completely quiescent. The observed "bunching effect" due to such thigmokinesis, results, in the case of the woodlice, in a decrease in waterloss from individual animals (Friedlander, 1963). It may therefore be interpreted as a strategy for reducing the effects of desiccation in these animals living high on the shore (see also Agnew, 1985 on Echinogammarus pirloti and E. obtusatus). Allee in Hurley (1968) also noted that such aggregations of terrestrial isopods normally collect at the moist end of a humidity gradient. This is also the case in Orchestia (Backlund, 1945; Williamson, 1951a).

The wrack banks inhabited by O. gammarellus are warmer than the ambient air temperatures thoughout the year; temperature variations being minimal in the deepest layers (Backlund, 1945; Moore and Francis, 1985a). This fact may be important to the survival of this non-hibernating animal (O. gammarellus is active throughout the winter (Bousfield, 1958)). During this study it was observed that even under extremely cold conditions when many Orchestia living beneath stones high on the beach had died, animals living beneath the strand line debris were still active and continued to feed.

Orchestia gammarellus has a wide tolerance of temperature; the lower and higher lethal temperatures being -6 to -3^{0}C and 37^{0}C respectively (Backlund, 1945; Moore and Francis, 1985a). These figures, however, do not allow for the possibility of seasonal acclimation that is known to occur in other talitrid species, eg. in the euterrestrial Arcitalitrus sylvaticus (Lazo-Wasem, 1984). In this species the upper lethal temperature in the summer is several degrees

higher than that observed during the winter. As yet there is no comparable information for the temperature tolerance of O. mediterranea. As data become available it may be found that temperature preferenda and tolerances will be shown to be important factors not just in delineating the different geographic distributions of both species but also in maintaining their respective vertical zonation patterns on the shore.

It should be noted that the recent reporting of Platorchestia platensis from the South east coast of England (Wildish and Lincoln, 1979) may have serious implications for local British populations of O. gammarellus. The two species co-occur on the European coast where P. platenis was apparently introduced in about 1860, and it has since supplanted O. gammarellus in some locations (Behbehani and Croker, 1982). The general competitive superiority of P. platensis over O. gammarellus has been attributed to the longer reproductive period of P. platensis and to its more rapid maturity, large brood size and large number of broods per breeding season (Dahl, 1946; Karlbrink, 1969).

2.2.4 ACTIVITY RHYTHMS AND ORIENTATION

Orchestia gammarellus exibits a circadian endogenous activity rhythm, browsing at night, at and above H.W.M. The rhythm has no endogenous tidal component for this species does not show a tidal migration onto the lower shore. In contrast, O. mediterranea (which lives below O. gammarellus on the shore) possesses endogenous rhythms of both circadian and tidal frequency (Wildish, 1970a). The persistence and precision of the locomotor rhythm and the definition of the nocturnal activity phase, however, are greater in burrowing species than in the more cryptozoic, non-substrate modifying (sensu MacIntyre, 1963) talitrids (Williams, 1983a). It is interesting that Duncan

(1969) has suggested that different locomotory patterns in euterrestrial talitrids may reduce competition between a pair of species which exhibit similar foraging patterns (as is most certainly the case for O. gammarellus and O. mediterranea).

Although talitrid amphipod activity is predominantly nocturnal, a time-compensated sun compass orientation mechanism has been demonstrated in O. mediterranea (Pardi, 1960) and several other littoral (substrate - modifying) amphipods (Pardi and Papi, 1952; Pardi and Grassi, 1955; Enright, 1961; Ercolini, 1964; Ercolini et al., 1983). Celestial orientation involving the moon has been demonstrated in many sandhoppers eg. Talitrus if not specifically in the Orchestia spp. presently under consideration (Papi and Pardi, 1955, 1959, 1963; Papi, 1960; Enright, 1961, 1972). However, direction finding may also depend to varying extents on humidity and the level of substratum moisture (Pardi and Papi, 1952; Van der Bercken et.al., 1967), on visual landmarks (Williamson, 1951b, 1954; Scapini and Bartolozzi, 1983), on prevailing winds (Pardi and Papi, 1952), on thigmotaxis (Enright, 1978) and on hydrostatic pressure (Enright, 1978). There is also some evidence that sandhoppers may use the slope of the beach itself to aid orientation (Craig,1973a, b).Ardense and Barendregt(1981) working with O. cavimana have established the roles of both magnetic and photo-orientation particularly correlated with pre-experimental waterloss. It has been found that humidity and salinity can act synergistically to influence sun compass setting (referred to briefly above) in Talitrus saltator (Scapini, 1978). A combination of low humidity and diluted seawater causes them to move from land toward the sea but when the salt content of the water reaches a critical level (between 3 and 5 ‰), Talitrus heads in the opposite direction. Orientation, although extensively examined in various sandy beach species (see Newell, 1979; Herrnkind, 1983 for

reviews of the mechanisms involved), has been little studied in O. gammarellus or O. mediterranea. This is probably due to the fact that these Orchestia species are comparatively "stationary" when compared with many of the sandhoppers which often traverse considerable distances in search of food (Dahl, 1946; Geppetti and Tongiorgi, 1967).

2.2.5 DISPERSAL

Orchestia gammarellus is dependent upon the sea for passive dispersal (Karlbrink, 1969). When placed in water it will cling to any floating object (Karlbrink, 1969; Wildish, 1970b; see also Bock, 1967 for details on P. platensis). Dahl (1946) suggested that, because many birds are seen to pick at the wrack beds, "one can well imagine that frightened Orchestia may leap onto them and seek protection among the feathers and are carried away for longer or shorter distances." Segerstrale et al. (1954) in Hurley (1968) have produced considerable geographical and experimental evidence for a theory of (aquatic) amphipod distribution involving transportation by birds. Such a method of dispersal, if it takes place at all in talitrid amphipods, could only be effective over short distances. Active dispersal on the other hand would appear to be insignificant in the determination of the distribution of O. gammarellus (Dahl, 1946; Karlbrink, 1969). The exception to this is found in the case of pressure being exerted by outside forces. For example, flooding of the habitat may result in the forced migration of large numbers of these animals (Schellenberg, 1942; Dahl, 1946; Miss C.H. Francis, pers. comm.). In a Swedish population of P. platensis, orientation is in a north-south direction (ie. along the coast) which Karlbrink (1969) suggested may be advantageous if this species is introduced into a new area. He also noted that this reaction was absent from O. gammarellus.

2.2.6 LOCOMOTION

Most talitrids are poor swimmers (Dahl, 1946). Some of the more terrestrial forms cannot swim at all (Lawrence, 1953; Hurley, 1968). Orchestia mediterrenea being eulittoral (Wildish, 1982a), however, is a notable exception: it appears to be very much at home in the water. It swims with the body fully extended and the antennae pointing forwards and lying parallel to one another. The peraeopods extend backwards and a strong pleopod beat (Walshe-Maetz, 1956) propels the animal forwards. A similar swimming behaviour has been described previously for Orchestia palustris (Smallwood, 1905). In contrast, when O. gammarellus is placed in water it is more inclined to walk than swim (Robertson, 1886). If forced to swim it does so by means of rapid flicks of the urosome. These flicks propel the animal forward in a series of movements which are both awkward and transitory. Swimming behaviour and ecological energetics of some talitrid species have recently been the subject of fairly extensive study by Vogel (1985a) as has the functional morphology of the musculature involved (Vogel, 1985b). Robertson (1886) wrote that O. gammarellus, "cannot live continuously in water though it looks at home for a time." However, both O. gammarellus and O. mediterranea can live indefinitely in well aerated water (32‰) between 10 and 20^{0}C (Reid, 1947; J.I.S. pers. obs.).

Terrestrial locomotion in O. gammarellus was first described by Chevreux (1888). Walking in both O. gammarellus and O. mediterranea does not appear to be very efficient; the laterally compressed body extends upward to a height that is just greater than the width between the peraeopods: because the centre of gravity is so high the animal is potentially unstable. Peraeopods 3-5 are modified in such a way as to provide stability while walking upright (Bowers, 1964) and are

specially strengthened for jumping (Charniaux-Legrand, 1952; Bulycheva quoted in Hurley, 1968). Shortening of the urosome and strengthening of the uropods is also associated with the jumping habit (Hurley, 1968; E.L. Bousfield in Kaestner, 1970 p. 478). When either species is disturbed animals begin to jump in a random fashion until they reach safety (Sars, 1890; Ruppel 1967). After 2-3 hops they will often curl up into a ball and feign death before the resumption of either walking or jumping behaviour. Death feigning is a common feature of many talitrid species (Holmes, 1903). The movement preference of Orchestia can be summarised as quiescent>walking>jumping (J.I.S. pers.obs.). The jump of O. cavimana, a species morphologically similar to O. gammarellus and O. mediterranea, has been studied by Bracht (1980b). He found that this species, in a jump lasting 350-400 msec., covered a distance of 18 cm, and achieved an average acceleration on take-off of 300 $m.s^{-2}$.

In O. gammarellus, there is no hardening of the cuticle by calcium carbonate deposition as is normally the case for more marine amphipods. This results in the integument being softer and more flexible than is the rule in amphipods (Reid, 1947). The weight loss involved is hardly sufficient for this change to be explained in terms of adaptation to the jumping habit. Reid (1947) suggested that this change may make the animal, "supple enough for crawling into dry apertures."

Although not as common a behaviour pattern as in T. saltator (Williams, 1983b) and other sandhoppers (Reid, 1947), burrowing has been observed in O. gammarellus both on the shore (Schellenberg, 1942; Den Hartog, 1964; J.I.S. pers.obs.) and in the laboratory (J.I.S. pers obs.). In the case of T. saltator it appears that burrow depth is regulated primarily by the requirement of the amphipods to achieve an

optimum humidity in the surroundings (Williams, 1983b).

2.2.7 REPRODUCTION AND DEVELOPMENT

Reproduction and development in the Talitridae has been the subject of extensive investigation. Williamson (1949a, 1953) has observed mating in O. gammarellus and O. mediterranea. The male will only mate with a fresnly moulted female. During copulation the female is carried beneath the male's body in such a manner that they can run and jump if necessary. She is supported chiefly by the male's second gnathopods, gripping one of her thoracic coxae. In O. gammarellus, the female is carried for about an hour, during which time copulation takes place four to five times. In O. mediterranea carrying is continued up until egg laying which can be up to 23 h after copulation. The time of egg laying is not determined by the time of copulation but by the time of moult. Egg laying always occurs 24 h after the moult in females of both species. Charniaux-Cotton (1957) has observed sterile interspecific mating between O. gammarellus and O. mediterranea.

Female O. gammarellus are found bearing eggs during the summer months (Den Hartog, 1964; Moore and Francis, 1986b; J.I.S. pers. obs.) from March to either September (Amanieu, 1967a,b; Williamson, 1949a) or October (Backlund, 1945). Dahl (1946) recorded a bimodal pattern in the number of gravid females in the summer months, which he attributed to precocious juvenile development. Charniaux-Cotton (1957) and Amanieu (1967a), however, suggest that this phenomenon was largely due to sampling error as it is unlikely, in their view, that O. gammarellus could obtain a gravid condition in three or four months. Williams (1978a), too, found that in Talitrus saltator, sexually differentiated juveniles did not contribute to the secondary breeding population during the summer breeding season. It has been reported for

a Mediterranean population of O. mediterranea though, that young animals born in the middle of the spring enter into reproductive activity by the end of the summer of the next year (Louis, 1977a,b). This discrepancy may either reflect a genuine difference between the species or it may be due to latitudinal difference. Despite the confusion, it may be said that such observed cycles in reproductive behaviour suggest the synchronisation of female behaviour in having a few broods in a good season.

Williamson (1954) was of the opinion that the breeding rhythm is partly inherent and partly controlled by climate; both egg laying and juvenile development are temperature dependent (Charniaux-Cotton, 1957; Bock, 1967). It has been suggested that a semi-annual species which produces two generations per year in the more southerly part of its range (eg. O. gammarellus), is probably annual towards its northern range limit (Wildish, 1982b). In this connection, a study of O. gammarellus from the extreme south-east coast of Iceland would be of great interest. The breeding season in O. mediterranea is similar to that mentioned above (Bate and Westwood, 1863; Ulianin, 1880; Williamson, 1949; Louis, 1977a,b).

Female Orchestia spp. carry between 8 and 25 eggs in the brood pouch (Williamson, 1949a; Morino, 1978; Wildish, 1982b), the single egg volume (as % body weight) being 0.056% in O. mediterranea and 0.114% in O. gammarellus (Wildish, 1982b). The eggs of O. gammarellus are purple (Bate and Westwood, 1863; Scott, 1956) as is the case in many other talitrid species (Rawlinson, 1937). The hatched young remain in the female marsupium for a few days orientated perpendicular to the longitudinal body axis. Their heads rest in the shallow depressions located between the body segments. When the adult is squeezed gently, the young are able to eject themselves from the brood pouch and hop to safety. The young are normally bright orange in

colour in both species (Bate and Westwood, 1863).

Orchestia gammarellus has a maximum life expectancy of 18 months (Charniaux-Cotton, 1957) when raised in the laboratory although Dorsman (1935) found that O. cavimana had a life span of approximately 12 months in the wild. Similarly, P. platensis can live for a period of about 12 months but in natural populations this figure is very often reduced by as much as half (Behbehani and Croker, 1982).

In O. gammarellus there is an initial rapid juvenile growth rate followed by a decrease in rate following sexual differentiation (Wildish, 1972). The development, growth and moult cycle of O. gammarellus and O. mediterranea has been described in great detail (Ulianin, 1880, 1881; Charniaux-Legrand, 1952; Charniaux-Cotton, 1957, 1960; Wildish, 1972). It is also similar to that of other talitrids (Dahl, 1946; Lafon, 1958; Williams, 1978a) and intertidal gammarid amphipods (Sheader and Chia, 1970) that have been studied. O. gammarellus exhibits a pattern of indeterminate growth with no terminal ecdysis (Charniaux-Cotton, 1957). Moulting frequency in both species is affected by various extrinsic factors such as temperature (Charniaux-Cotton, 1957), the salinity of the food/substratum (Wildish, 1970c) and photoperiodism (Kurata, 1962). Charniaux-Cotton (1957) has also described selected features of the moult in O. gammarellus which enable rapid determination of the moult stage. Post-moult (Stages A-B) can be recognised by the presence of a soft exoskeleton. Intermoult (Stage C) is defined as the time when there is no obvious empty space beneath the cuticle of the second gnathopod. This period is very variable in the Crustacea as a whole. It ranges from less than 24 h to two or three years, the latter being the case in some of the larger decapods (Hartnoll, 1982). In O. gammarellus this period lasts about three weeks. Charniaux-Cotton divides the

pre-moult condition into two stages. If there is a space present between the old and the new cuticle, or if there is an uncuticularised claw present, then this is taken as early pre-moult (Stages D0-1). Late pre-moult is detected by the cuticularisation of the new claw.

The sex ratio for both species is normally 1:1 (Wildish, 1970a) except in certain circumstances such as the colonisation of unstable habitats where a biased sex ratio may be of adaptive value (Wildish, 1971). Intersexuality is very common in O. gammarellus (Charniaux-Cotton, 1957) as in many other talitrids (Boulenger, 1908; David, 1936; Charniaux-Cotton, 1960). This particular phenomenon has been investigated extensively by Ginsburger-Vogel (see Ginsburger-Vogel and Charniaux-Cotton, 1982 for references and review). On the Isle of Cumbrae, the greatest number of intersex individuals were taken from above the M.H.W.M. They were characterised by the presence of both oostegites and gnathopods, the latter being markedly different from the normal appendage.

Morino (1978) and Wildish (1979, 1982b) have considered the evolutionary adaptations and ecology of reproduction in various talitrid species.

2.2.8 FEEDING

Talitrids are important in the degradation of stranded kelp (Brenner et al., 1976; Lopez, 1977; Lopez et al. ,1977; Cammen, 1980; Griffiths and Stenton-Dozey, 1981). Orchestia gammarellus will eat all dead organic matter indiscriminately, provided that it is not too hard or dry (Backlund, 1945; Bock, 1967). Perkins (1974) recorded an algal intake equivalent to 3.42% of the wet weight of laminarian algae.Orchestia body weight^{-1}.day^{-1}.. Although they consume large amounts of material, the greater percentage of it is not digested (Backlund, 1945). Some work carried out at Garelochhead (Perkins,

1974) has confirmed these observations and shown also that Orchestia may live naturally in leaf-litter and consume cardboard as readily as more natural materials. This cosmopolitan voraciousness extends even to "a handkerchief, which a lady let fall amongst them, (which) was soon reduced to a piece of open work by the minute jaws of these small creatures" (Bate and Westwood, 1863). Some recent work by Moore and Francis (1985b) on feeding in O. gammarellus has shown that while the animal consumed mainly plant (as opposed to animal) material, the overall composition of the material varied depending upon food item availability on the shore.

Gut morphology and the physiology of digestion in talitrids, and especially O. gammarellus has been the subject of extensive investigation (Agrawel, 1961a,b, 1962a,b,c, 1964a,b,c,d; Wildish and Poole, 1970; Shyamasundari, 1973, 1979, 1981). Some talitrids eat their own exuviae as well as dead of their own kind (Bowers, 1964; Miss C.H. Francis, pers.comm.). Bate (1862) observed a number of hoppers, "congregating together beneath a stone, busy in devouring a common earthworm". Unfortunately he does not mention whether or not the earthworm was alive at the time.

2.2.9 ASSOCIATIONS

Orchestia gammarellus is often found in association with the sea-slater, Ligia oceanica and the fully terrestrial woodlouse Porcellio scaber (Den Hartog, 1963; Scott, 1956; J.I.S. pers.obs.) as well as with

various insect and arachnid species (Backlund, 1945; Scott, 1956; Roth and Brown, 1976; Simpson, 1976; J.I.S. pers.obs.). Orchestia mediterranea is commonly found in association with Echinogammarus marinus (Chevreux and Fage, 1925) and E. pirloti (J.I.S. pers.obs.).

The nocturnal habit of both O. gammarellus and O.mediterranea as well as many other semi-terrestrial amphipods (Bowers, 1964), helps them to avoid surface predators, mainly birds eg. the Chough (Roberts, 1982), the Lyre Bird (Searle, 1928), the Rock Pipit (Gibb, 1955), the Ringed Plover (Duncan, 1910) and species of Sandpiper (Reid, 1947). They are also preyed upon by various intertidal beetles (Bate and Westwood, 1863; Richards, 1982, 1983, 1984), centipedes (Roth and Brown, 1976) and spiders (Duncan, 1969; Roth and Brown, 1976; Moloney and Nicolson, 1984).

As is the case with many terrestrial arthropods, talitrids are infested with various parasitic mites and Protozoa (Trouessart, 1902; Canaris, 1962; Felgenhauer, 1979; Kitron, 1980; Duncan, 1981; Overstreet, 1983; Moore and Spicer, 1985; P.G. Moore pers.comm.). Such infestation has been frequently noted on O. gammarellus (Trouessart, 1902; J.I.S. pers. obs.). As a consequence of these and other "fouling organisms", many species have evolved a very intricate series of stereotyped grooming movements (Holmquist, 1982, 1985). There is very little information, however, on grooming in O. gammarellus and O. mediterranea. The haplosporidian Marteilia sp. is endoparasitic in O. gammarellus and is reported to produce a temperature-sensitive feminising effect (Desportes and Ioim, 1981). Bate and Westwood (1863) have noted the occurrence of phosphorescence in O. gammarellus. This phenomenon has also been reported in other talitrids (Bousfield and Klawe, 1963). In O. gammarellus, it appears to be related to a disease caused by micro-organisms of the genus Diplobacterium, a condition which is often fatal (Giard, 1889; Giard and Billet, 1889). Some closely related species also act as intermediate hosts for some chicken tapeworms (Alicata, 1936).

2.2.10 ECONOMIC IMPORTANCE

Until fairly recently, Orchestia spp. have been regarded as of little economic importance (Reid, 1947). In the last few years, however, an interest has been developing in the use of Orchestia (and some Talorchestia sp. see Venables 1981, a,b,c) as a source of protein in both domestic animal feed-stuffs (Moore and Francis, pers.comm.) and in compounded diets in aquaculture (Lachaux et al., 1984). As long ago as 1947, Reid had suggested that, for farms in close proximity to the seashore, these animals could serve as a source of additional protein for feeding chickens and he outlined a simple method for their capture and preparation. He also noted in passing that even beachfleas accidently ingested by unwary persons may be far from unpalatable! A method for the extraction of talitrids from beach wrack has recently been outlined by Ciavatti (1983). Only Roubald (1948/49) has recorded sandhoppers biting a human hand, so O. gammarellus would not appear to pose any threat to human beings!

Some of the more terrestrial amphipods such as Arcitalitrus sylvaticus and A. dorrieni (see below) are often found in human habitations (particularly after heavy rainfall) almost to the extent of being regarded as pests (Mallis, 1942, 1960; Hunt, 1925; Richardson, 1980). On occasion, O. gammarellus has invaded the Marine station at Port Erin on the Isle of Man (Walker, 1895). More recently the same species was accidently introduced into households in a Greenock housing estate (P.G.Moore,pers. comm.) and also a home in Rothesay (Miss M. Bain,pers. comm.). Moore (1984) also identified O. gammarellus from under the carpet of a home in Glasgow. This semi-terrestrial amphipod, however, does not warrant the same pest status as more terrestrial synanthropic species in the genus Arcitalitrus.

It should be noted finally that pollution has an affect on the natural history of these animals. Oil spillages have been responsible

for mass mortality in populations of Platorchestia platensis (Boyle, 1969). It is also known that many semi-terrestrial amphipods accumulate trace metals (Bender, 1975; Rainbow and Moore, in press) but information on toxicity and sub-lethal effects is limited.

2.3 ORCHESTIA CAVIMANA

Orchestia cavimana (Heller, 1865) is a fully fresh-water talitrid (Hynes et al., 1960) which was first recorded in this country from beneath stones above H.W.M on the River Thames at Richmond in Surrey (Gordon, 1944; see also Cain and Cushing, 1948). It is known to occur as far north as Leicester (R.R. Harris, pers. comm.) and Yorkshire (Fryer, 1950, 1951; Curry et al., 1972). Schellenberg (1940) has synonymised O. cavimana with O. bottae. This entity, reported as either O. cavimana or O. bottae, is widespread in fresh-water habitats thoughout Europe (Heller, 1865; Lienhart, 1913; Schlienz, 1922; Spandl, 1924; Chevreux and Faye, 1925; Giltay, 1927; Dudich, 1927; Bayard and Carayon, 1946; Beckmann, 1941; Karaman, 1965; Kinzelbach, 1965, 1972; Karlbrink, 1969; Bracht, 1980a). Notes on its natural history and ecology exist (Dorsman, 1935; Dahl, 1946; Den Hartog, 1963; Reid, 1947) although there is but one quantitative study in the literature (Wildish, 1970b). This partially terrestrial hopper is considered by Bousfield (1984), to be a vestige of an invasion by Tethyan beachflea progenitors onto land in the temperate north Atlantic.

Locomotion in O. cavimana has recently been studied by Bracht (1980) and Vogel (1985a,b). Little attention however, has been paid to the physiology of this species. The information that exists, is concerned only with orientation (Ardense and Barendregt, 1981; Scapini and Bartozelli, 1983; see also previous section) and locomotor

rhythmicity (Wildish, 1970a). There is one account which compares aerial vs aquatic respiration in O. cavimana (Ivlev and Suschenya, 1961).

Individuals used in the respiration and gill studies described in chapters 3 and 4 respectively were collected from the banks of the River Thames at Isleworth Ait (O.S. grid ref. TQ 167 761). The animals were uncommon on the local riverbank,apparently due to the general unsuitability of micro-habitats available, with the exception that is of the location sampled. Orchestia cavimana was observed in the laboratory eating both Beech and Sycamore leaves, which had been left lying on top of their native substratum. The soft parts of the leaves were consumed first leaving just the midrib and stem untouched. Despite being collected during the summer months (July, 1985), no ovigerous females were noted.

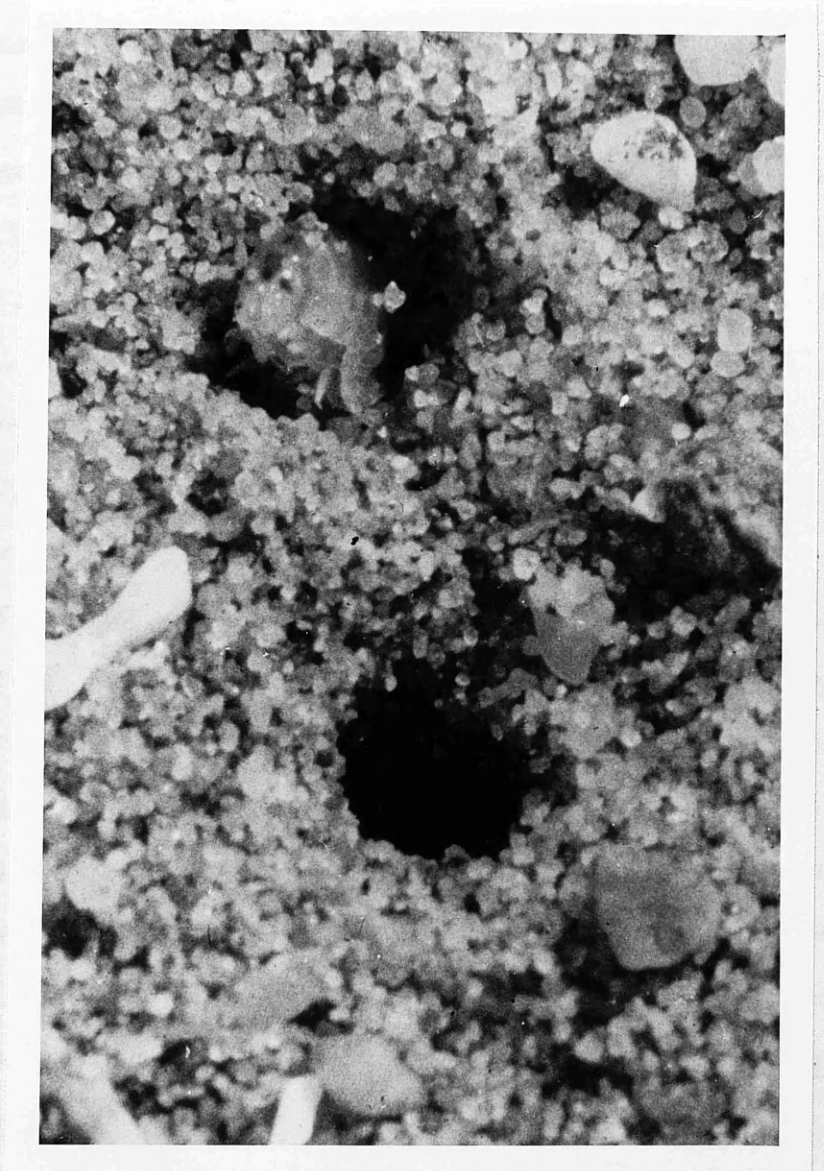
2.4 TALITRUS SALTATOR AND TALORCHESTIA DESHAYESII

The sandhoppers examined in these studies were taken from around the H.W.M at two locations on the Isle of Cumbrae in the Firth of Clyde; Ballochmartin Bay (O.S. NS 17 318437) and Fintray Bay (O.S. NS 17 318437) (Fig. 2.8). Talitrus saltator (Montagu) is abundant on most clean sandy beaches (Fig. 2.6c) at or just above the H.W.M. Individuals are normally a green/buff or fawn colour with a black dorsal line. Noticeably more robust than Orchestia spp., the male and female can grow up to 25 and 18mm long respectively. During the day, they can be found either within their burrows in the sand (Fig. 2.9) or beneath wrack and other debris cast up by the tide. At night they leave their burrows and move up and down the beach in search of food. As was discussed above, they are able to recognise and return to the upper beach by taking into account the slope of the beach and the

FIG. 2.8 Fintray Bay on the West side of Great Cumbrae Island.

Both T. saltator and T. deshayesii were concentrated in a zone between the lowest and highest wrack strings in the picture (5.6.85). Animals used in the gill studies were collected from this beach.

FIG 2.9 Talitrus saltator emerging from burrow at H.W.M. at Fintray Bay, Great Cumbrae Island. Note the sand grains adhering to the animals' integument.



outline of the top of the beach or other notable objects silhouetted against the sky.

Talitrus saltator reproduces from mid-April to late-September (Williamson, 1949a) and appears to be largely quiescent during the winter months. Animals uncovered 0.5m below the surface on Ballochmartin Bay in December, 1984, took from 1 - 5 minutes to overcome their general lethargy on being disturbed. The well-being of a sandhopper population is dependent on the continued existence of the beach. On a visit to Skye in August, 1983, the Sandy beach at Glen Brittle on the West side of the island, was observed to support a large population of T. saltator. On a return visit exactly two years later, it was found that the sandy beach, presumably after storm damage, had been superceded by a coarse grade, boulder beach. Sandhoppers were noticeable by their absence, although O. gammarellus was present at H.W.M.

The biology and habits of Talitrus saltator are considerably better documented than any of the other species presently considered (Robertson, 1886, 1888; Sars, 1890; Jancke, 1926; Schellenberg, 1928; Verway, 1929; Dahl, 1946; Pallaualt, 1954; Den Hartog, 1963) as is its ecology and physiology (Williamson, 1949a, 1951a,b, 1954; Williams, 1978a,b, 1980a,b,c, 1981, 1982a,b, 1983; Ardense, 1978, 1980; Bercken et al., 1967; Storch and Burkhardt, 1984; Bregazzi, 1972; Bregazzi and Naylor, 1972; also see first section on O. gammarellus and O. mediterranea).

In general, the ecology of Talorchestia deshayesii (Audouin, 1826) is similar to that of T. saltator. Consequently they are often found on the same beach, as is the case on the Isle of Cumbrae and other Scottish localities (Fig. 2.6d). Dahl (1946), however, found that T. deshayesii preferred much finer sand when compared with T. saltator. Reid (1947) found that T. deshayesii extended further up-

shore than T. saltator, so that it was possible to find Talorchestia, "under stones high enough above H.W.M. to be sharing its habitat with woodlice and earthworms." On the continent, T. deshayesii is active from the end of March to the beginning of November (Den Hartog, 1963). Personal observations on the Isle of Cumbrae in general support this finding.

While the female superficially resembles T. saltator, the second gnathopod in male Talorchestia deshayesii is strongly sub-chelate, the species being markedly sexually dimorphic. Talorchestia deshayesii is normally smaller than T. saltator, and is sand coloured with dark brown broken stripes on the dorsal surface. A full description of its burrowing habits is given by Reid (1938). Some other literature on its general habits and ecology does exist but information is somewhat incomplete (Bate and Westwood, 1863; Dahl, 1946; Reid, 1929, 1947; Den Hartog, 1963; Karlbrink, 1969; Louis, 1980). With the exception of but four papers (Marchionni, 1963; Pardi and Grassi, 1955; Williamson, 1951a; Williams, 1982b), the species' physiology is totally unexamined.

Pseudorchestoidea brito, Bousfield, 1982 (= Talorchestia brito Stebbing) was not examined during the present study. What is known of its biology is given by Vader (1968, 1970).

2.5 TALORCHESTIA SP. A

Individuals of Talorchestia sp. A., used in the gill area analysis (Chapter 4) were collected from McFaddin beach, 15 miles west of the Sabine Pass and 35 miles from Port Arthur, Texas, U.S.A. The animals were moderately common at H.W.M., beneath rotting seaweed on fine grained sandy beaches. The animals were kindly collected on 25.8.84 by Mr A. Ford and Mr and Mrs Charles Elliot. The air

temperature at the time was 85^0F and the relative humidity was 95%. The animals when disturbed, were able to jump 0.3 - 1.2m into the air and were captured by "swatting" the animals in mid-air and collecting them by hand when they feigned death (A. Ford, pers. comm.).

Examination by Dr. P.G. Moore and myself verified that the animals belonged to the genus Talorchestia; the exact species however, was uncertain. Dr. E. Lashley on behalf of Dr. E.L. Bousfield of the National Museum of Natural Sciences, Ottawa, Canada, in a personal communication, stated that he had nearly completed a paper on sandhoppers of the southeastern and Gulf coasts of the United States, containing several new species which previously would have been included in the genus Talorchestia. However, as these large, heavy bodied boreal and Virginian American Atlantic species are nearly as different from the true Talorchestia as are members of the counterpart Pacific coast genus Megalorchestia (Brandt) from the more warm temperate and tropical Pseudorchestoidea (Bousfield), Bousfield and his co-workers have felt it necessary to erect a new genus to encompass them (Ophthalmorchestia). It is thus very likely, although not confirmed, that Talorchestia sp. A is in fact Ophthalmorchestia barbarae n.sp. (Bousfield).

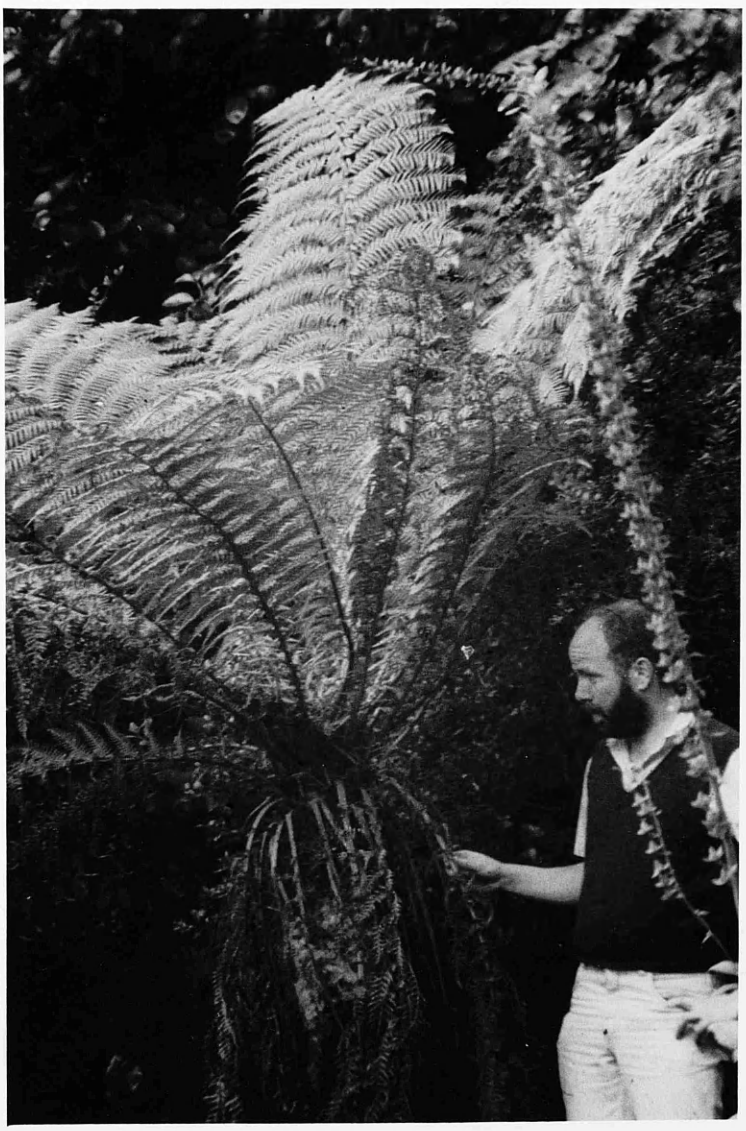
2.6 ARCITALITRUS DORRIENI

The euterrestrial landhopper Arcitalitrus dorrieni was first recorded in the United Kingdom from ornamental gardens on the Isles of Scilly (Hunt, 1925). Since then it has been recorded free-living from several mild temperate sites in both England and Ireland (Rawlinson, 1937; Ingle, 1958; Murphy, 1973, 1974; Richardson, 1980). Dr. R.J. Lincoln (pers. comm.) has 22 specimens lodged in the British Museum (Nat. Hist.) by Mr. J. Rundle from collections made by a Nature

Conservancy Council survey of Colonsay in June, 1978. The present author and Dr. P.G. Moore on a visit to Colonsay (8th - 10th May, 1985), investigated 65 sites encompassing a variety of microhabitats on both Colonsay and adjacent Oronsay, to both confirm the record and determine the present distribution of this species. Arcitalitrus dorrieni was found to be widely distributed on Colonsay, among deciduous leaf litter in wooded areas. It also occurred, however, in bracken litter and to a lesser extent on scrub wasteland. There would appear to be a clear concentration of landhoppers in the leaf litter accumulations adjacent to Colonsay House - presumed to be the original locus of colonisation. The species was absent from nearby Oronsay, presumably due to the lack of tree cover there. A full account of the status of A. dorrieni on Colonsay can be found in Moore and Spicer (1986).

In the Scilly Isles, A. dorrieni exhibited a preference for tree-fern litter (Dicksonia antarctica) (Richardson, 1980), a plant that was also introduced into Colonsay in the last century (Moore and Spicer, 1986). It was conceivable therefore that populations of landhoppers existed at other Scottisn locations, mild enough to support this sub-tropical plant. Two species of tree fern, Dicksonia antarctica and D. squarrosa occur in the grounds of Inverewe gardens in the north-west corner of the Highlands (Fig. 2.10). An extensive search of leaf litter accumulations at Inverewe, particularly around these ferns and other plants native to Australia and New Zealand (including Eucalyptus coccifera, E. pauciflora and E. dalrympleana), however, failed to reveal any landhoppers. These tree ferns are also to be found in the Botanic Gardens, Glasgow. The closely related Talitroides alluaudi has been reported from these gardens in the past (Palmen, 1949). Extensive investigation of the areas surrounding the Australian and New Zealand plants in the Glasgow hot houses, however,

FIG. 2.10 Dixonia antartica. Inverewe gardens.



also failed to reveal any amphipods. At present then, Colonsay would seem to be the only Scottish locality now supporting a terrestrial amphipod fauna.

Animals used in the respiration and gill studies (Chapters 3 and 4) were collected from sycamore leaf litter accumulations in the grounds of Colonsay House (O.S. NR 394971) . They were returned to the laboratory on the day of capture, in a large plastic bag containing both earth and leaf litter. To extract living A. dorrieni from the leaf litter a flooding technique was employed (Ciavatti, 1983) It has previously been reported that these animals are very susceptible to "drowning" (Duncan, 1969). However, up to 48 hours after total inundation of the leaf litter, animals could be seen walking on debris just below the water surface. When a number of individuals were placed in a beaker of fresh (tap) water, they were seen to walk from one wall of the container to another without too much sign of stress. When disturbed, however, this amphipod did appear to be incapable of any swimming movements whatsoever. In fact, when animals crawling on top of inundated leaf litter were startled, they readily submerged themselves by crawling to the lower reaches of the container. When forcibly submerged though, the mortality rate of A. dorrieni was very high.

Arcitalitrus dorrieni is more brittle than its supralittoral zone counterparts. During handling, it was not unusual to break one or more peraeopods or an antenna. The integument of A. dorrieni thus resembles more closely the heavily calcified marine amphipods rather than its supralittoral talitrid relatives, eg. Orchestia or Talitrus.

CHAPTER THREE

RESPIRATION IN AIR AND WATER OF SELECTED TALITRID SPECIES.*

3.1 INTRODUCTION

The ability of intertidal invertebrates to respire in both air and water is well documented (see Newell, 1973, 1979 for reviews). The aerial and aquatic oxygen consumption rates of various intertidal gastropods have been studied extensively in relation to their ecological distribution on the shore (Davies, 1966; Micallef, 1967; McMahon and Russell-Hunter, 1977; Houlihan, 1979; Innes *et al.*, 1984). In many cases, the aerial/aquatic oxygen consumption ratio for a particular species increases with the degree of emersion and therefore can be correlated with the animals vertical zonation on the shore (Houlihan and Innes, 1982).

Similar trends have been found in a recent study on three decapod crustacean species representing aquatic, semi- and fully terrestrial habitats (O'Mahoney and Full, 1984). Houlihan and Innes (1984) have also shown that this pattern of aerial/aquatic oxygen consumption is similar in both active and inactive decapods.

Little attention has been paid to this aspect of the ecophysiology of talitrid amphipods. They are of special interest as presumably they rely on the same respiratory structure, the gill, in both media. Recent studies have shown that two semi-terrestrial beachfleas, *Transorchestia chiliensis* and *Chroestia lota* can maintain their aerial oxygen consumption rates when submerged (Marsden, 1984, 1985). Ivlev and Suschenya (1961), however, found that although oxygen consumption rates in air and water were similar for subtidal *Gammarus locusta*, in the semi-terrestrial *Orchestia cavimana*, aerial respiration was three times the aquatic rate. Most other studies on talitrid respiration,

* Much of this chapter is embodied in a paper by Spicer and Taylor, accepted for publication by *J. exp. mar. Biol. Ecol.*

have been concerned primarily with aerial oxygen consumption as related to energy budgets (Clark, 1955; Richardson and Morton, 1986), the effects of season and latitude (Edwards and Irving, 1943; Venables,1981a,c;Van Senus,1985) and the demonstration of circadian rhythms in oxygen consumption (Williams, 1981, 1982a; Moore and Francis, pers.comm.). There are however, two papers concerning the effect of hypoxia on O. mediterranea under aquatic conditions (Walshe-Maetz, 1952, 1956).

In the present study, the aerial and aquatic oxygen consumption of Orchestia mediterranea, O. gammarellus, O. cavimana and Arcitalitrus dorrieni, species of differing degrees of terrestrial adaptation were examined over the ecologically realistic temperature range 5 to 30^{0}C. Also included in this chapter are the results of a short experiment to assess the tolerance to submersion of each species.

Preliminary investigation of aerial oxygen consumption in Orchestia gammarellus with and without gills was also attempted. It was hoped that data obtained from such an experiment would be helpful in elucidating the role of the gills in oxygen uptake and would identify, in a more quantitative fashion, the possibility of extrabranchial routes of oxygen exchange.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Orchestia gammarellus and O. mediterranea were collected by hand from beneath stones and amongst the wrack beds at and below H.W.M. (H.W.M.) at Farland Bight on the Isle of Cumbrae (O.S. Grid Ref. NS 17 324412). Orchestia cavimana was collected from H.W.M. on the River

Thames at Isleworth Ait (O.S.Grid. Ref. TQ 167 761) and Arcitalitrus dorrieni from Sycamore leaf litter on the island ofColonsay, inner Hebrides (see chapter 2 for more details on O. cavimana and A. dorrieni collection sites). All animals were brought back to the laboratory in large polythene bags containing their natural substratum. Animals were maintained in a constant temperature room at 10 ± 1^{0}C for a period of six days. During this time they experienced a 12:12 LD regime. Animals were fed on decaying seaweed or deciduous leaves as appropriate. Measurements of oxygen consumption were carried using large male animals (1.0 - 28.0 mg dry weight) with the exception of the smaller A. dorrieni, (weight range 0.3 and 8.0 mg dry wt.). Each animal was used only once. Only animals corresponding to intermoult C (Charniaux-Cotton, 1957) were used.

3.2.2 Immersion tolerance experiment

For the tolerance experiments, amphipods were separated into two length categories; group A > 10mm body length and group B < 10mm body length (measured from the tip of the telson to the basis of the antenna). All Arcitalitrus used belonged to group B. Both males and (non-ovigerous) females were considered. 20 individuals of each species (with the exception of O. cavimana where n = 10) were exposed to humid aerial conditions at 10 and 20^{0}C throughout the experiment. The control chambers were 100 ml glass crystallising dishes, covered with a perforated plastic petri dish lid (which prevented the animals escaping). Each dish contained a sheet of folded filter paper soaked in sea water (32‰) (for O. gammarellus and O. mediterranea) or tap water (for O. cavimana and A. dorrieni). 20 individuals of each species were also immersed in sea water or tap water (as appropriate). The animals were placed in covered 100 ml crystallising dishes (to prevent evaporation) each containing 80 ml of constantly aerated

water. In both aerial and aquatic experiments, the dishes were checked regularly and any mortalities noted. Both the immersion medium and filter paper were replaced every 3 days and dead animals removed. Animals were fed during the experiment with fresh wrack or leaf litter as appropriate.

3.2.3 Aquatic oxygen consumption

Rates of aquatic oxygen consumption (10 - 30^{0}C) were measured by enclosing individual animals in darkened glass bottles (Volume = 25 ml) with gauze lids. A piece of large mesh gauze was inserted into each bottle to provide a substratum to which the animals could cling. It was found that providing such a substratum reduced their activity and ensured that the majority of readings obtained were for inactive animals. The bottles were submerged in a large tank of filtered seawater (salinity = 32‰) in the case of O. gammarellus and O. mediterranea or filtered tapwater in the case of O. cavimana and A. dorrieni (A. dorrieni was also tested in 30% sea water). The water in these tanks was constantly aerated and the animals were left for 3 h to acclimatize to the experimental conditions. The temporary gauze lids were then carefully replaced by screw-top lids after the initial Po_2 of the water had been measured. This was done by removing a 1 ml water sample from the bottle and injecting it into a thermostatted electrode holder (at the experimental temperature) containing an oxygen electrode (Radiometer E5046) connected to an oxygen meter (Strathkelvin Instruments, Glasgow). Three empty bottles were also run as controls to estimate the rate of background respiration. At the end of the experimental period (1 - 3 hours), each bottle was quickly inverted several times to ensure thorough mixing and the Po_2 of the water determined as above. The rate of oxygen depletion in each bottle was then calculated. To ensure that the rates of oxygen consumption

were unaffected by hypoxia, the oxygen tension of the water in which the animals were kept was never allowed to fall below 120 Torr. Finally the animals were removed, killed and dried at 60^0C for two days prior to weighing, to an accuracy of 0.1mg, on a Mettler Type H 16 balance.

3.2.4 Aerial oxygen consumption

Rates of aerial oxygen consumption at different temperatures (5 - 35^0C) were measured using a Gilson differential respirometer. Each respiration flask (volume = 7 and 15 mls) contained only one animal, a filter paper wick saturated in 10% KOH solution (to absorb Carbon dioxide), and a small piece of filter paper previously soaked in filtered seawater or tapwater as appropriate. This was found to reduce both locomotor activity and the mortality of the animals. Animals were kept for 12 h at the experimental temperature before recordings commenced. Respiration rates were measured at either 30 or 60 minute intervals over a 5 - 6 h period. At the end of each experiment the animals were killed, dried and weighed as outlined above.

The rates of oxygen consumption were expressed in microlitres per milligram dry weight per hour, R_s ($\mu l.O_2.mg^{-1}.h^{-1}$). The notation follows Sutcliffe (1984). All oxygen consumption data were corrected for S.T.P. and regressed on a double log plot against the dry body weight of the animal. Covariance analysis was used to compare data obtained for each species.

3.2.5 Gill excision

All the gills from 30 male Orchestia gammarellus were removed under a binocular dissecting microscope (x10) using two pairs of very fine forceps. There were no apparent ill-effects of the operation and all the experimental animals survived the duration of the experiment (6 h., 100% R.H.). Aerial oxygen consumption of animals with excised

gills was measured as outlined above. For each Gilson "run" (n = 12) at temperatures between 10 and 20^{0}C, 6 control animals with intact gills were also included.

3.3 RESULTS

3.3.1 Immersion tolerance

In all species examined, little mortality resulted from containment in humid aerial conditions at both experimental temperatures within both size ranges. All animals were maintained for a period exceeding 6 weeks and would probably have survived much longer had not the experiment been terminated. Table 3.1 shows the survivorship of the four species when immersed. All three Orchestia species were able to survive immersion at both experimental temperatures almost indefinitely. Indeed, O. mediterranea was maintained submerged in the laboratory for a period of 9 months until the animals eventually died due to neglect. In the case of A. dorrieni, however, the animals were unable to tolerate immersion stress for any considerable time. All animals were dead after 12 h at 20^{0}C with 50% survivorship (LT_{50}) at around 7hrs. Survivorship increased with decreasing temperature. The survival ofimmersed A. dorrieni was also greatly enhanced if provision was made for the animals to leave the water periodically.

3.3.2 Weight exponent

A linear relationship between weight specific oxygen consumption, (R_S) and body dry weight, (W) was obtained when plotted after a double logarithmic transformation and could be described by a regression

TABLE 3.1 Tolerance to immersion in water (*O. gammarellus* and *O. mediterranea* - sea water 30 ‰ and *O. cavimana* and *A. dorrieni* - tap water) by four talitrid species. Numbers of survivors where initial n = 20 in each case.

TEMPERATURE = 10°C

	Time immersed						
species	3hr	6hr	9hr	12hr	1 day	7 days	14 days
O. gammarellus	20	20	20	20	20	20	19
O. mediterranea	20	20	20	20	20	19	19
O. cavimana	20	20	20	20	20	18	18
A. dorrieni	20	18	17	9	0	-	-

TEMPERATURE = 20°C

	Time immersed						
species	3hr	6hr	9hr	12hr	1 day	7 days	14 days
O. gammarellus	20	20	20	20	19	19	17
O. mediterranea	20	20	20	20	20	20	19
O. cavimana	20	20	20	20	20	19	16
A. dorrieni	17	11	4	0	-	-	-

equation in the form

$$\text{Log } R_S = \text{Log } a + b \text{ Log } W$$

where Log a is a constant and b is the regression coefficient. All of the regression equations linking rates of oxygen consumption and dry body weight (Table 3.2) had statistically significant correlation coefficients with the exception of O. gammarellus and O. mediterranea at 5^0C. As in most other crustaceans (Newell, 1973, 1979; Sutcliffe, 1984), R_S decreases with increasing body weight. The value for b at 10^0C was between -0.478 and -0.141 for all of the talitrid species examined (Table 3.2). Covariance analysis indicated that the differences in the value of b between the species was not significant. The combined regression coefficient, b = -0.231 of the talitrid species studied is comparable with the similar figures of -0.248 and -0.266 for the freshwater species Gammarus pulex (10^0C) and G. fossarum (12^0C) respectively (Rumpus and Kennedy, 1974; Pieper, 1978 both quoted in Sutcliffe, 1984) and -0.271 for the intertidal Echinogammarus obtusata (Agnew, 1985). The measurements presented in this chapter were made under minimal but uncontrollable locomotor activity and could therefore considered as representing the animals "routine metabolic rate".

3.3.3 Aerial and aquatic oxygen consumption at 10^0C

In Figure 3.1, the regression lines (described by the equations in Table 3.1) for both aerial and aquatic oxygen consumption at 10^0C in O. gammarellus and O. mediterranea are plotted against body dry weight. Covariance analysis showed no significant difference ($P > 0.05$) between aerial and aquatic R_S in O. gammarellus. In O. mediterranea however, both the slope and elevation of the aerial and

TABLE 3.2 Regression constants for lines of best fit of R_S (Y) on body dry weight (X) in Orchestia gammarellus, O. mediterranea, O. cavimana and Arcitalitrus dorrieni in air and water: equation in the form log Y = log a + b log X, values of 'a' are log numbers. Values for the correlation coefficient (r) and the number of animals used (n) are also given.

O. gammarellus

	aerial				aquatic			
T(^{0}C)	a	b	r	n	a	b	r	n
5	0.299	-0.849	0.27	18	-	-	-	-
10	0.210	-0.367	0.86	39	-0.015	-0.147	0.81	16
15	0.358	-0.334	0.79	40	0.341	-0.320	0.79	21
20	0.332	-0.188	0.69	32	0.436	-0.328	0.86	18
25	0.511	-0.254	0.74	19	0.594	-0.368	0.93	17
30	0.803	-0.472	0.83	17	-	-	-	-

O. mediterranea

	aerial				aquatic			
T(^{0}C)	a	b	r	n	a	b	r	n
5	0.320	-0.991	2.10	18	-	-	-	-
10	0.017	-0.240	0.87	21	0.014	-0.141	0.84	18
15	0.387	-0.337	0.80	27	0.320	-0.299	0.86	17
20	0.527	-0.380	0.78	24	0.430	-0.310	0.91	13
25	0.630	-0.330	0.83	14	0.742	-0.427	0.90	17
30	0.790	-0.478	0.84	21	-	-	-	-

TABLE 3.2 (continued)

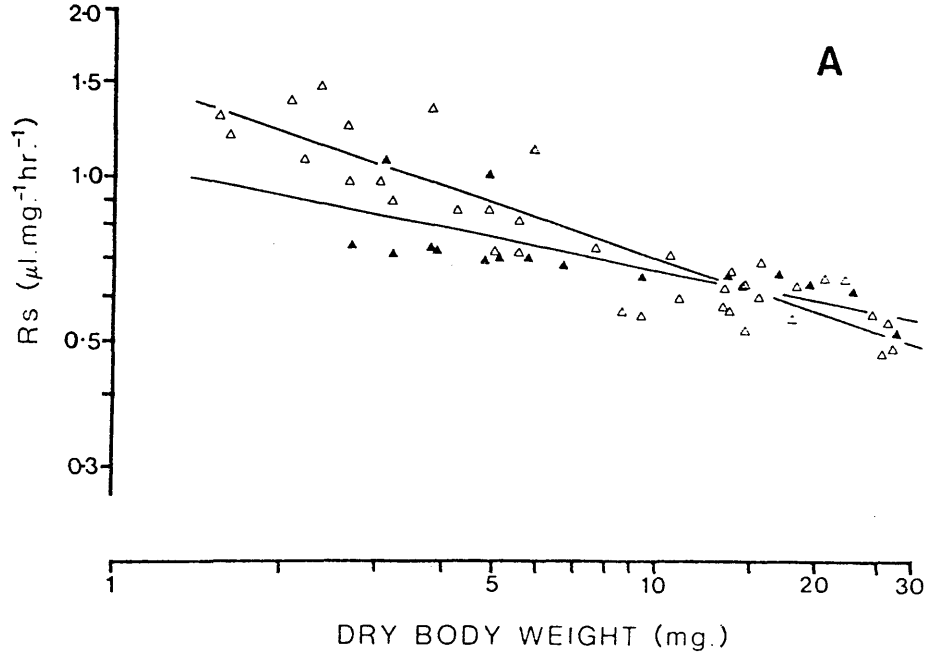
O.cavimana

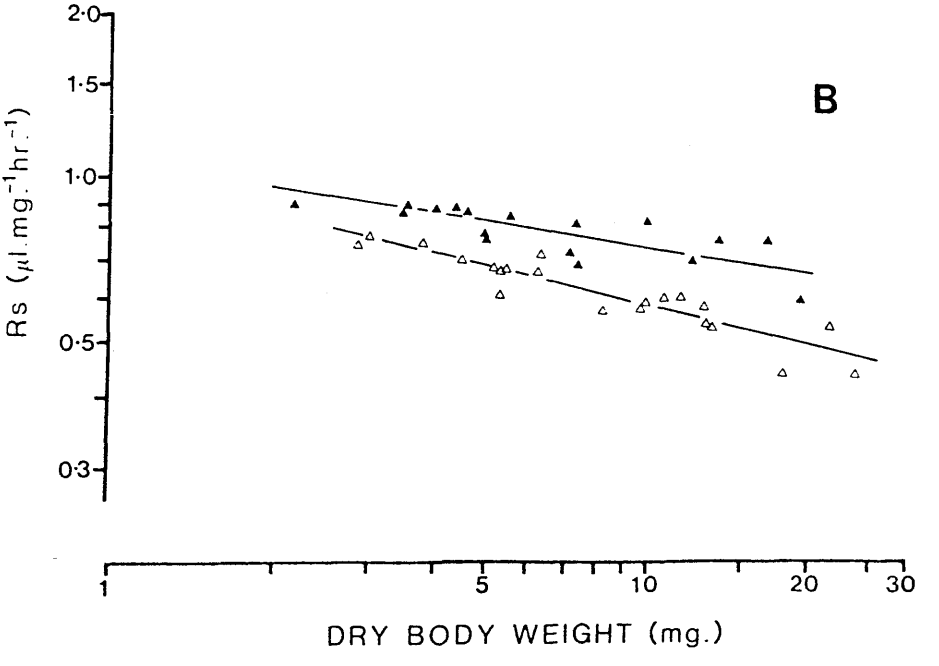
	aerial				aquatic			
T(^{0}C)	a	b	r	n	a	b	r	n
10	0.078	-0.240	0.73	12	-0.214	-0.143	0.79	12
15	0.217	-0.124	0.78	12	0.204	-0.425	0.82	11
20	0.350	-0.117	0.79	11	0.475	-0.451	0.81	11
25	0.504	-0.233	0.84	12	-	-	-	-

A. dorrieni

	aerial				aquatic			
T(^{0})	a	b	r	n	a	b	r	n
10	0.255	-0.243	0.78	16	-0.203	-0.318	0.78	20
15	0.374	-0.255	0.88	18	-	-	-	-
20	0.947	-0.576	0.71	17	-0.023	-0.345	0.88	11
25	0.954	-0.537	0.74	20	-	-	-	-
30	0.930	-0.570	0.78	17	-	-	-	-

FIG. 3.1 The relationship between weight specific oxygen consumption in air (△) and water (▲) at 10^0C against dry body weight in O. gammarellus (A) and O. mediterranea (B).


A
2·0
1·5
1·0
0·5
0·3
Rs (µl.mg.⁻¹hr.⁻¹)
1
5
10
20
30
DRY BODY WEIGHT (mg.)

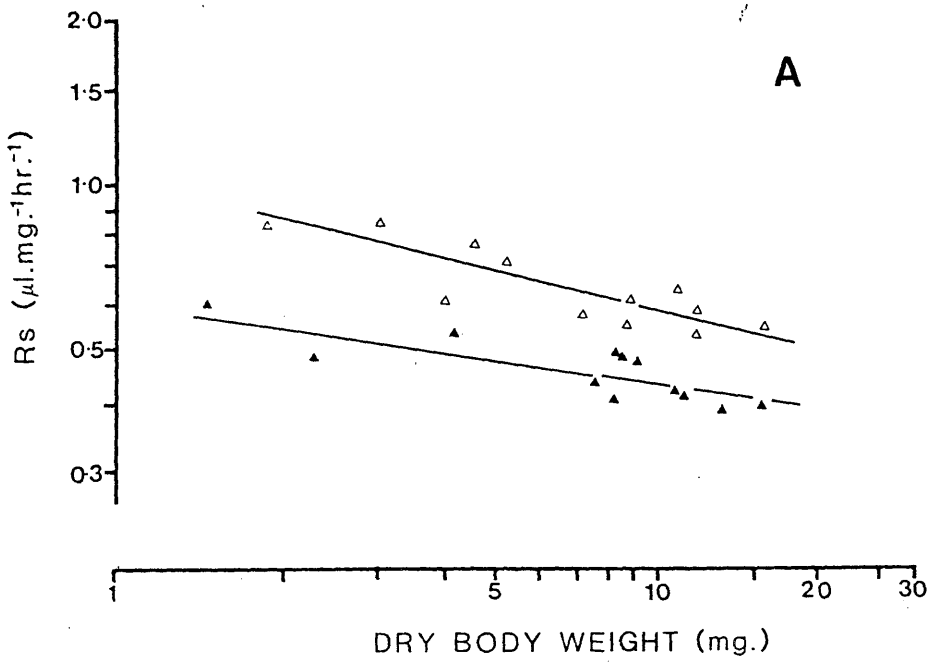

B
2·0
1·5
1·0
0·5
0·3
Rs (µl.mg.⁻¹hr.⁻¹)
1
5
10
20
30
DRY BODY WEIGHT (mg.)

aquatic regression lines were significantly different from one another (F_s=4.79; d.f.= 1,35 $P<0.05$: F_e=48.77; d.f.=1,36 $P<0.01$). Similar plots of aerial/aquatic oxygen consumption (regression lines described by the equations given in Table 3.1) for O. cavimana and A. dorrieni are given in Figure 3.2. It was shown using covariance analysis that although the slopes of the aerial and aquatic R_s for each species were not significantly different, the elevation did differ significantly ($P < 0.01$). The rate of oxygen consumption in water for both species was considerably reduced compared to those in air. Covariance analysis indicated that there was no significant difference between the slopes of the lines describing aerial R_s (Combined regression coeffient = -0.315) with the exception of O. mediterranea (O.g/O.m., $F_e(1,56)$ = 18.184; $P < 0.01$: A.d./O.m., $F_e(1,34)$ = 6.16; $P < 0.05$: O.c/O.m., $F_e(1,35)$ = 13.55; $P < 0.01$) and A. dorrieni (A.d./O.g., $F_e(1,53)$ = 9.13; $P < 0.01$). In fact, the aquatic R_s of O. mediterranea was similar to the aerial R_s of the other species studied. An intraspecific comparison of the aerial/aquatic R_s for each of the species showed that, with the exception of O. gammarellus, R_s in water was significantly different from R_s in air ($P < 0.01$).

3.3.4 Effect of temperature on R_s

R_s values for 'standard' 5mg and 20mg animals of each species were calculated from the regression equations in Table 3.2. The relationships between the rates of aerial and aquatic oxygen consumption and temperature for all four species are illustrated in Figure 3.3. In both air and water, R_s for all the species studied was temperature dependant. There was very little difference in aerial oxygen consumption rates between the Orchestia spp.

FIG. 3.2 The relationship between weight specific oxygen consumption in air (Δ) and water (▲) at 10^0C against dry body weight for O. cavimana (A) and A. dorrieni (B).


A
2·0
1·5
1·0
0·5
0·3
Rs (µl.mg.⁻¹hr.⁻¹)
1
5
10
20
30
DRY BODY WEIGHT (mg.)

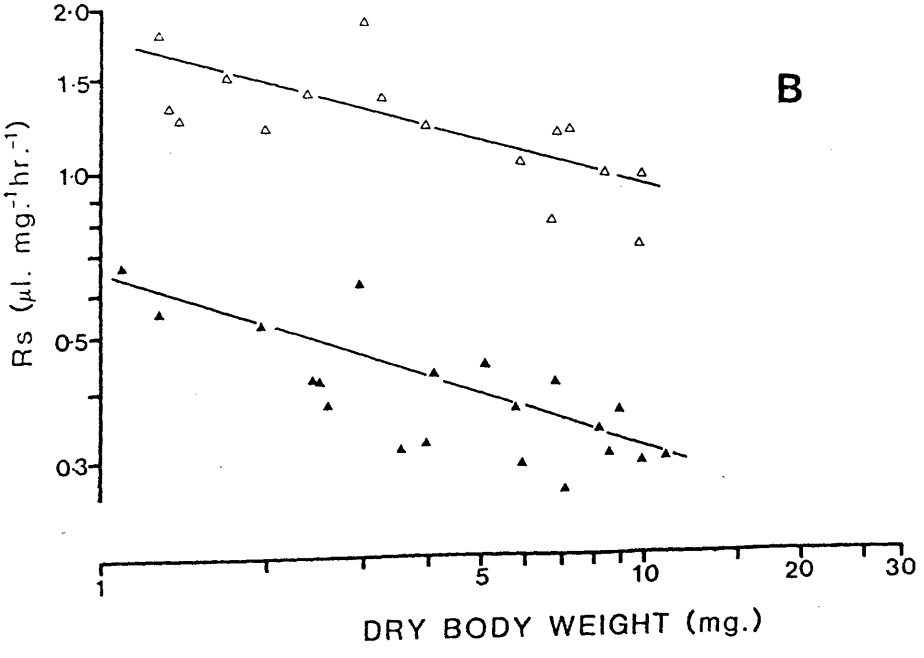

B
2·0
1·5
1·0
0·5
0·3
Rs (µl. mg.⁻¹hr.⁻¹)
1
5
10
20
30
DRY BODY WEIGHT (mg.)

FIG. 3.3 The relationship between oxygen consumption in air (open symbols) and water (closed symbols) (Y) and temperature (X). Oxygen consumption rates for standard animals were calculated from regression equations in Table 3.2. squares = 20mg dry body weight: triangles = 5mg dry body weight.

A. C. gammarellus
B. C. mediterranea
C. O. cavimana
D. A. dorrieni

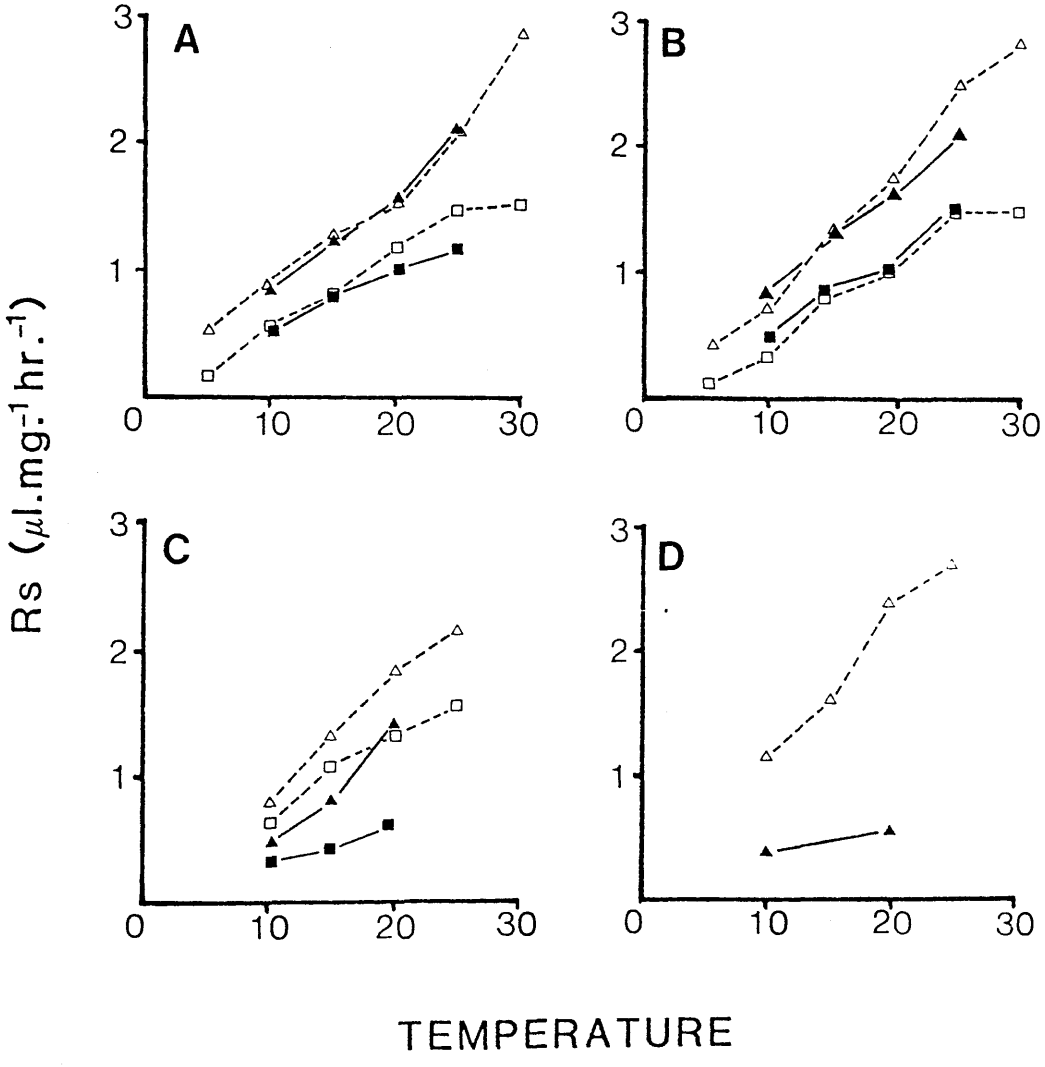

A
B
C
D
3
2
1
0
10
20
30
Rs (μl.mg.-1hr.-1)
TEMPERATURE

TABLE 3.3 Q_{10} values for O. gammarellus, O. mediterranea, O. cavimana and Arcitalitrus dorrieni (acclimated to 10^{0}C) in air and water.

		Rs aerial		Rs aquatic	
Species	Temp (^{o}C)	5mg	20mg	5mg	20mg
Orchestia gammarellus	5-10	3.97	13.0	-	-
	10-15	2.21	2.31	2.96	1.88
	15-20	1.42	2.11	1.51	1.42
	20-25	1.85	1.55	1.80	1.69
	25-30	1.89	1.02	-	-
Orchestia mediterranea	5-10	3.85	22.00	-	-
	10-15	3.00	3.59	1.96	1.96
	15-20	1.65	1.13	2.03	2.09
	20-25	1.43	1.26	2.87	2.09
	25-30	1.57	1.36	-	-
Orchestia cavimana	10-15	1.59	2.28	2.78	1.27
	15-20	3.28	2.24	3.17	3.02
	20-25	1.39	1.48	-	-
Arcitalitrus dorrieni	10-15	2.01	1.94		
	15-20	2.00	2.00	1.56*	1.92*
	20-25	1.30	1.18		

* A. dorrieni, aquatic respiration: temperature range = 10-20^{0}C

In Orchestia mediterranea the rates of aquatic oxygen consumption were greater than aerial rates over the temperature range 10 - 20^{0}C, although this feature was not so pronounced in the 5 mg animals. There was no difference in the rate of aerial oxygen consumption when measured at 25 and 30^{0}C. However, none of the species examined including O. mediterranea was able to maintain an aquatic rate of oxygen consumption at these elevated temperatures and the mortality rate was observed to be high. In O. gammarellus, aerial and aquatic rates of oxygen consumption were similar over the range 10 - 20^{0}C, but at25^{0}C the aerial:aquatic ratio increased markedly from 1 to 1.65. At temperatures higher than 25^{0}C, the rate of oxgen consumption of O. mediterranea in air does not increase markedly. For O. cavimana and A. dorrieni, between 10 and 30^{0}C, the aerial rates of oxygen consumption were similarto that of O. gammarellus. The rates of aquatic oxygen consumption, however, remain consistently low, with neither species able to withstand water temperatures in excess of 25^{0}C.

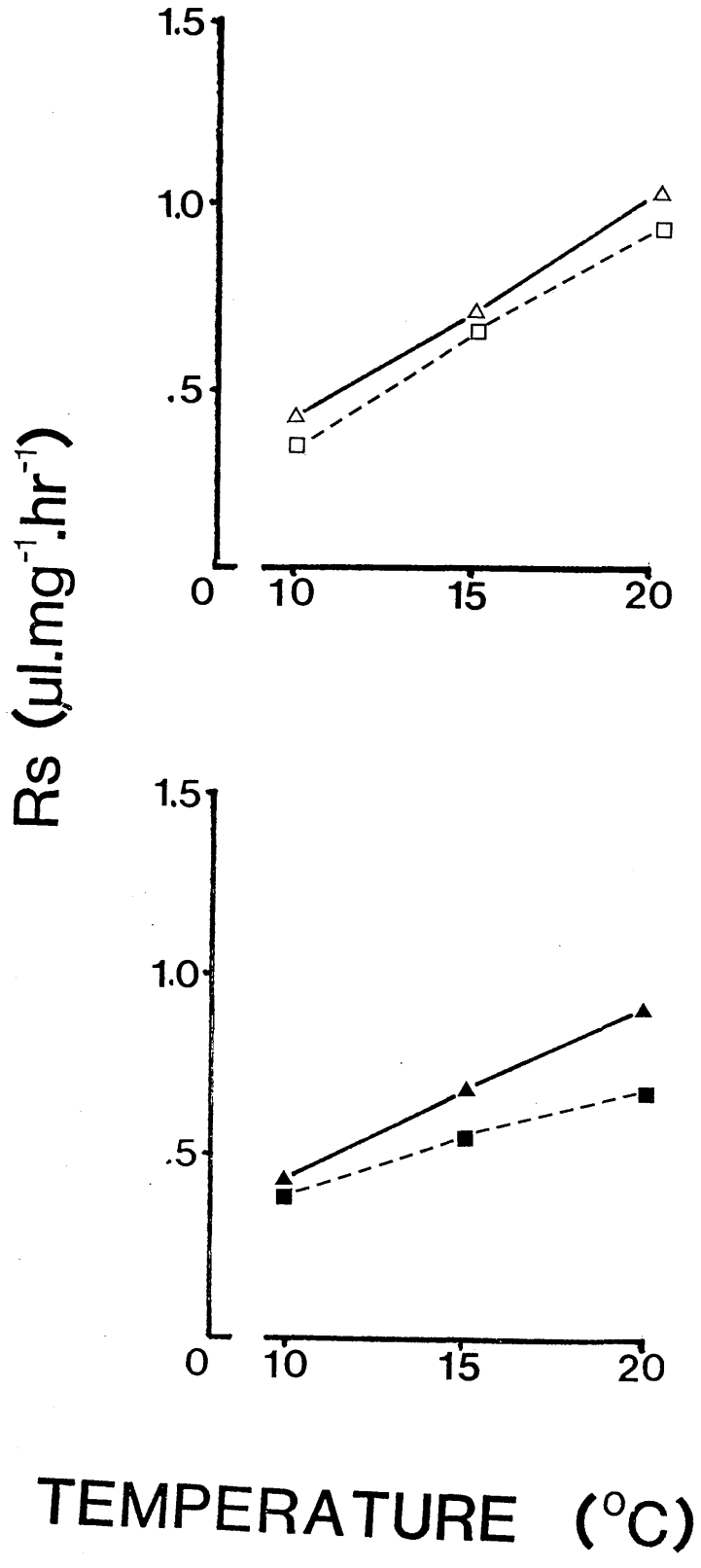
The temperature coefficient, Q_{10} was calculated for each 5^{0}C interval between 10 and 30^{0}C in all of the species (Table 3.3). The Q_{10} varied inversely with temperature in all of the species within the temperature range studied.

3.3.5 Gill excision

Figure 3.4 shows the aerial and aquatic oxygen consumption rates of a standard 20mg O. gammarellus with and without gills, over the temperature range 10 - 20^{0}C. Although at 10^{0}C the group with their gills excised had a lower R_S than the control animals (12%), the difference was not statistically significant at P = 0.05. Most animals were able to survive without their gills for as long as did the controls. At temperatures greater than 15^{0}C, however, animals without

Fig. 3.4 The relationship between oxygen consumption in air (open symbols) and water (closed symbols) (Y) and temperature (x) of O. gammarellus with (triangles) and without (squares) gills. Oxygen consumption rates were calculated for a 20 mg dry body weight, standard animal from the following regression equations.

aerial/with gills	10^0C	Y = -0.27X + 0.07
	15^0C	Y = -0.24X + 0.16
	20^0C	Y = -0.23X + 0.31
aerial/without gills	10^0C	Y = -0.38X + 0.01
	15^0C	Y = -0.06X + 0.05
	20^0C	Y = -0.22X + 0.27
aquatic/with gills	10^0C	Y = -0.17X - 0.18
	15^0C	Y = -0.18X + 0.06
	20^0C	Y = -0.27X + 0.31
aquatic/without gills	10^0C	Y = -0.22X - 0.13
	15^0C	Y = -0.13X - 0.10
	20^0C	Y = -0.09X - 0.05


1.5
1.0
.5
0
10
15
20
Rs (µl.mg⁻¹.hr⁻¹)
TEMPERATURE (°C)

gills had a significantly lower aquatic R_S than control animals and were unable to survive prolonged exposure to water temperatures in excess of 20^0C.

3.4 DISCUSSION

3.4.1 Effect of Immersion on Oxygen consumption and survival

Table 3.4 is a compilation of the data on the ratios of aerial:aquatic respiration obtained during the present study together with comparable values from studies on other talitrids. This information has been placed within the eco-morphological framework proposed by Bousfield (1984). There are, however, only three of Bousfield's four groups represented; we lack data for the palustral talitrids (Group I). The ability of talitrid amphipods to maintain an aerial rate of oxygen consumption following immersion can be roughly correlated with their degree of emancipation from the aquatic environment. This conclusion is in full agreement with previous work carried out on a number of crustacean and molluscan groups (Taylor and Davies, 1981; Houlihan and Innes, 1984; O'Mahoney and Full, 1984; Powers and Bliss, 1983; McMahon and Russell-Hunter, 1977; Innes et al., 1984). Within the beachflea (Group II) and landhopper (Group IV: both 1 and 2) groups, there is a trend toward loss of bimodal respiration. This is most marked in the landhopper species represented.

The eulittoral O. mediterranea is immersed at each high tide (Wildish, 1982a)and so a retention of bimodal respiration was not unexpected. Orchestia gammarellus is also able to respire equally well in air and water. Such an ability to maintain an aquatic rate of oxygen consumption in air has also been reported in a subtropical

TABLE 3.4 Aerial:aquatic oxygen consumption ratios for a number of talitrid amphipods presented within the eco-morphological scheme suggested by Bousfield (1984). Ratios have been calculated for a 'standard'5 mg dry weight animal. Species marked with an asterisk were unable to maintain an aerial oxygen consumption when immersed. References:- (1) Present study, (2) Marsden, 1986, (3) Marsden, 1985, (4) Ivlev and Suschenya, 1961, (5) Pallaualt, 1954.

GROUP II (Beachfleas)		GROUP III (Sandhoppers)	
Orchestia mediterranea	0.85 (1)	Talitrus saltator	1.00 (5) [?]
Chroestia lota	1.01 (2)		
O. gammarellus	1.01 (1)		
Transorchestia chiliensis	1.33 (3)	GROUP IV (Landhoppers) IV.1	
O. cavimana	1.67 (1)	Arcitalitrus dorrieni *	3.23 (1)
O. bottae	2.30 (4)		

beachflea, Chroestia lota (Marsden, 1985) and in some other crustaceans such as the shore crab, Carcinus maenas (Newell et al., 1972), the semi-terrestrial crab, Cardisoma guanhumi (Taylor and Butler, 1978; O'Mahoney and Full, 1984) and the freshwater crayfish Austropotamobius pallipes (Taylor and Wheatly, 1980).

It has been suggested that there is little difference between the rate of oxygen consumption of intertidal animals in air and water when measured at comparable activity levels (Newell et al., 1972; Newell, 1973). However, as in the decapods studied by Houlihan and Innes (1984), this was clearly not the case in Arcitalitrus dorrieni or Orchestia cavimana. In these amphipods there was a marked suppression of oxygen consumption upon immersion. Ivlev and Suschenya (1961) found that in O. cavimana (as O. bottae), aerial respiration rates were twice those in water. This is far greater than was observed during the present study. The elevated respiration rates in air observed by Ivlev and Suschenya (1961), may have been accentuated, however, by unconstrained movement of the animals within the experimental vessels.

Dorsman (1935) kept O. cavimana alive for many months under conditions of forced immersion whereas in Orchestia patersoni and Makawe hurleyi, prolonged immersion eventually resulted in death (Duncan, 1969, and pers. comm.). The fact that all species presently examined, with the exception of A. dorrieni, can remain alive indefinitely in well-aerated water (a feature common to most supralittoral talitrids, see Smallwood, 1903, 1905; King, 1913; Pallaualt, 1954; Bowers, 1964; Marsden 1980, 1985; Moore and Francis, 1986 despite some reports to the contrary, eg. Robertson, 1888 and Bulycheva, 1957 as quoted in Hurley, 1968) would indicate that, as in

the case of the fully terrestrial decapods Gecarcinus lateralis (Taylor and Davies, 1981) and Ocypode saratan (Eshky, 1985), A. dorrieni has lost the ability to sustain prolonged oxygen uptake in water. This may be related to the loss and reduction of pleopods (pleopod 3 and 1 + 2 respectively) in A. dorrieni (Hunt, 1925). Although displaying values for aerial:aquatic R_S comparable to that of A. dorrieni, O. cavimana possesses well developed pleopods. In aquatic amphipods, the pleopods maintain a ventilatory current over the gills (Lincoln, 1979), therefore any reduction in these structures will presumably impair oxygen uptake in water, though not necessarily in air.

In many semi-/fully terrestrial decapods and isopods, reduction in the ability to sustain an aerial rate of respiration when submerged is often linked to profound changes in the nature of the gas exchange organs (Powers and Bliss, 1983). In the Talitridae, however, the reason for this reduction in aquatic respiration is far from apparent. It may be linked to both reduction in gill area, known to be a feature of many talitrids (see chapter 4), and/or limitations imposed by the oxygen transporting system.

In many decapod species there is a differentiation of the gill tissue into osmoregulatory and respiratory regions. Should this be true of amphipods, it may also help to reconcile the occurrence of the largest aerial:aquatic oxygen consumption ratio in A. dorrieni, the talitrid with the greatest gill area per unit weight of those studied. Further investigation of the comparative histology and histochemistry of amphipod gills is required before the validity of this hypothesis can be assessed.

3.4.2 Aerial respiration

Respiration in air by sessile inhabitants of the intertidal zone

involves a compromise between the need to ensure access of air to the respiratory surfaces, and the reduction of evaporative water loss which affects animals living at the higher levels of the shore (Newell, 1979). Talitrid amphipods, however, have overcome this difficulty by inhabiting wrack beds or leaf-litter accumulations with high interstitial relative humidity (Hurley, 1968; Moore and Francis, 1985a). Edney (1960) suggested that the ready availability of oxygen and the low density of air permits rapid and prolonged locomotion and subsequent elevated levels of oxygen consumption in terrestrial species. This predicted elevation in oxygen consumption was not found in any of the talitrids examined perhaps with the exception of A. dorrieni. Due to the smaller size of this species compared with the remaining species, however, it is perhaps unwise to regard this as a genuine increase in metabolism associated with the colonisation of land. The R_S of the remaining species, where comparable, was similar to the aquatic R_S of some more fully marine amphipods (Walshe-Maetz, 1952, 1956; Opalinski, 1982; Sutcliffe, 1984; Agnew, 1985) as well as that of other beachflea and sandhopper genera.

3.4.3 Effect of temperature on oxygen consumption

The aerial and aquatic oxygen uptake of all species studied was temperature dependent, a feature common to all other semi- and fully terrestrial amphipods examined to date (Marsden, 1984, 1985; see Introduction for references). Marsden (1984) noted that while plateaux of temperature independence have been found following thermal acclimation in the isopods Ligia oceanica and Sphaeroma rugica (Newell et al., 1976; Marsden, 1979), no such metabolic response was observed following laboratory acclimation of Transorchestia chiliensis. This is also true of the four species examined here.

Although the terrestrial environment is characterised by greater

extremes of temperature than the marine environment, talitrids inhabit an environment which is to some extent 'buffered' from these variations (Moore and Francis, 1985a). This 'buffering' capacity, however, diminishes as the volume of wrack decreases. Animals living beneath stones or within small wrack 'strings' will, therefore, be more susceptible to temperature stress than inhabitants of wrack beds (Backlund, 1945).

There is a considerable intraspecific variation in the values of Q_{10} which, together with differences in acclimation temperature, makes meaningful comparisons with data on other talitrids very difficult. Both the sublittoral amphipod Gammarus oceanicus (Halcrow and Boyd, 1967) and the supratidal tropical talitrid Talorchestia margaritae (Venables,1981a) have Q_{10} values in excess of 2. Q_{10} values > 2 over the temperature range 5 - 25^{0}C have also been calculated from Agnew's (1985) data relating to the eulittoral amphipods Echinogammarus pirloti and E. obtusatus. This reduction in the sensitivity of R_s to changes in temperature - often associated with the colonisation of the littoral/supralittoral zone - is very difficult to determine in the amphipods considered above, although there is a noticeable decrease in the Q_{10} value with an increase in temperature in all of the species examined.

Burggren and McMahon (1981), working on a range of hermit crabs from sublittoral, littoral and supralittoral habitats found that, over the normal environmental temperature ranges for these species, Q_{10} values for oxygen consumption determined for the littoral crabs were significantly lower than those for sublittoral or supralittoral species belonging to the same family; they also noted that a high Q_{10} for acutely determined oxygen consumption was not particularly disadvantageous for supratidal animals where environmental temperature changes may be much more gradual and attenuated. Such a

differentiation cannot be made on the basis of these present results for one eulittoral, two supralittoral and one euterrestrial amphipod in which, as noted above, there was very little difference between Q_{10} values over the corresponding temperature range. Great care must therefore be taken in formulating generalities, linking the temperature sensitivity of talitrid respiration with apparent habitat temperature regimes. It is clear that more data are required on both aquatic and fully terrestrial amphipod species before this can be attempted.

The high Q_{10} value, found for example for the aerial respiration of O. gammarellus and O. mediterranea between 5 and $10^{0}C$, was also noted in the supralittoral hermit crabs studied by Burggren and McMahon (1981). When these animals were exposed to such low temperatures, R_S was reduced dramatically, leaving these crabs "semi-torpid" and thus impairing foraging activity and the ability to avoid potential predators.

The onset of stressful conditions at the upper limit of thermal tolerance of O. gammarellus and O. mediterranea (30 - $35^{0}C$; Backlund, 1945; Moore and Francis, 1986a: J.I.S. unpubl. obs.) is often responsible for a decrease in the resting rates of aerial oxygen consumption at higher temperatures. This phenomenon has also been recorded in other eulittoral and supralittoral amphipods. None of the amphipods studied experience aquatic temperatures greater that $25^{0}C$. Not suprisingly, very few animals of any of the species considered here were able to survive prolonged aerial exposure (>8hrs) at $30^{0}C$. It is notable that in these semi- and fully terrestrial amphipods it was impossible to sustain aquatic oxygen consumption at temperatures above $25^{0}C$. This is undoubtedly related to the acquisition of terrestrial habits in these animals although the constraints involved

have not been elucidated.

3.4.4 Gill excision

It has been reported for both Talitrus saltator and an unnamed fully terrestrial landhopper that approximately half of the gas exchange that takes place does so at the gills (Pallault, 1954; Hurley, 1968). It was found in this study, however, that gill excision in O. gammarellus did not significantly affect the oxygen consumption of the animal at 10^0C although at 20^0C the oxygen consumption rates of animals with excised gills were significantly lower than those of the control animals. The answer to this apparent contradiction may be found in a consideration of the experimental techniques or the experimental temperature used in each case. It would appear that both Pallault and Duncan physically blocked the gills with an oxygen impermeable substance eg. paint, wax. Perhaps the reduction in oxygen consumption observed may have been a consequence of a trauma related to handling and/or toxicity in the method used to prevent gas exchange occurring at the gills.Williamson (1951a) found when the gills of O. gammarellus were blocked with paraffin wax, that the animals did not live any longer than a few hours. This is in marked contrast to the present study in which animals with excised gills, used in the aerial respiration experiments, showed no ill-effects on (or subsequent to) removal at relatively low ambient temperatures. This was not the case however when the experimental animals were submerged in sea water at temperatures in excess of 15^0C, indicating that the gills themselves may play a very important role in aquatic gas exchange. In air these animals can maintain an aerial rate of oxygen consumption via extra-branchial routes of gas exchange at a wide range of environmental temperatures but this ability decreases as temperature increases. The function and the significance of gill morphology relationships is considered more fully both in the next chapter and in chapter 7.

CHAPTER 4

A COMPARATIVE STUDY OF THE GILL AREA RELATIONSHIPS IN SELECTED TALITRID AMPHIPODS.*

4.1 INTRODUCTION

Although oxygen availability is greater in the aerial environment, terrestrial animals must still maintain moist respiratory surfaces (Edney, 1960). Thus the interdependence of respiration and water balance will presumably influence the adaptive radiation of a given group. Among the amphipods, the Talitridae is the only family which has truly terrestrial representatives (see chapters 1 and 2). Some of the intertidal gammarids exhibit a limited ability to survive in moist air (Lagerspetz, 1963) but it would be misleading to refer to them as terrestrial (Powers and Bliss, 1983).

Many terrestrial isopod and decapod Crustacea show morphological adaptations for aerial gas exchange. Although some species of woodlice continue to use their gills in gas exchange, in the more terrestrial species, gas exchange takes place within tubular invaginations (pseudotrachae) of the pleopods (Edney, 1968). Similarly in many terrestrial crabs, oxygen exchange takes place across the walls of the heavily vascularised branchial chambers (Greenaway, 1984) supplementing, and in some cases replacing the gills as the site of gas exchange. In land crabs, the gills themselves show various degrees of reduction in area and number which has been correlated with the move onto land (Little, 1983). Such specific respiratory modifications as occur in the isopods and decapods are absent from both semi- and fully terrestrial amphipods (Hurley, 1959). Whilst amphipods have retained a basic gill structure, it has recently been demonstrated

* Much of this chapter is embodied in a paper by Spicer J.I. and A.C. Taylor (1986) A comparative study of the gill area relationships in some talitrid amphipods. J. Nat. Hist., 20, 935-947.

that there is a reduction in the number and surface area of gills in the supralittoral O. gammarellus compared with that of other more marine amphipods (Moore and Taylor, 1984). The authors suggested that this reduction in gill area in O. gammarellus may be related to the acquisition of terrestrial habits. In contrast, it is commonly held that landhopper species exhibit an increase in their overall gill area (Matsuda, 1982). To my knowledge, however, a quantitative assessment of the gill area relationships of any fully terrestrial amphipod has not been published.

During the present study, the gill characteristics of talitrid amphipods taken from a number of different habitats were examined. The species chosen have colonised a variety of environments from marine littoral and supralittoral to fresh-water and fully terrestrial habitats. Both the gill morphology and distribution have been described. The total gill area was related to the body weight of seven different species (see below for details) to clarify the extent to which the Talitridae as a family are characterised by a reduction in gill area and number.

4.2 MATERIALS AND METHODS

4.2.1 Animal material

Orchestia gammarellus, O. mediterranea, Talitrus saltator and Talorchestia deshayesii were all collected from their respective habitats on the Great Cumbrae Island in the Firth of Clyde (lat. 55° 44'N; long. 4° 54'W). Orchestia cavimana was collected from the banks of the River Thames at Isleworth Ait (O.S. Grid ref. TQ 167 761). Arcitalitrus dorrieni was collected from sycamore leaf litter on the Isle of Colonsay (O.S. Grid ref. NR 394 971; Lat. 56° 07'N; long. 6° 18'W) (cf. Moore and Spicer, 1986). All British animals were

transported live back to the laboratory where they were maintained in leaf litter, wrack etc. according to habitat. Talorchestia sp. A was collected from McFaddin beach, 15 miles west of Sabine Pass and 35 miles from Port Arthur, Texas, on the Gulf of Mexico (lat. 29° 30'N; long.93° 53'W). These animals were preserved in 10% formalin, 1% glycerol diluted with 34‰ sea water. In some species, the total gill area of both preserved and fresh material were compared to validate the inclusion of the Talorchestia sp. A material. No significant difference was found between the data obtained from both groups.

4.2.2 Gill area measurements

Gill area measurements were carried out using the method of Moore and Taylor (1984) which involved drawing each of the excised gills, mounted in the appropriate medium without a coverslip, using a camera lucida. Each set of drawings was calibrated using a stage micrometer and the areas were calculated using a digitising pad interfaced to a Commodore PET microcomputer. Total gill area was expressed as the sum of the individual areas (x2) and regressed after double logarithmic transformation on body dry weight using the method of least squares. The body weight of the dissected animals was estimated by interpolation from the regression equation fitted to the log length/ log dry weight data for each of the species (Table 4.1).

4.3 RESULTS

4.3.1 Body length/ Body weight relationships

Regression equations of log body weight versus log body length in O. mediterranea, O. cavimana, A. dorrieni, Talitrus saltator and Talorchestia sp. A (see Table 4.1) were shown by covariance analysis,

TABLE 4.1 Regression constants for lines of best fit of dry body weight (Y) to body length (X) in the amphipod species investigated: equation in the form of log Y = log a + b log X, values of 'a' are log numbers.

Species	Regression constants a	b	Correlation coefficient (r)	n
Orchestia gammarellus	-1.87	2.72	0.976	20
Orchestia mediterranea	-1.78	2.43	0.949	37
Orchestia cavimana	-1.63	2.29	0.889	32
Talitrus saltator	-1.32	2.29	0.966	24
Talorchestia deshayesii	0.08	0.99	0.998	19
Talorchestia sp. A	-1.15	1.79	0.883	12
Arcitalitrus dorrieni	-2.32	2.63	0.688	19

not to be significantly different either in slope or elevation ($P = 0.1$) (Combined regression coefficient = 2.362). In the case of O. gammarellus, however, although the slope is similar to that found for the species listed above, the difference in elevation is highly significant ($P < 0.01$ in each case). This is because individuals of O. gammarellus are shorter per unit weight than any of the other talitrids investigated. The regression line for Talorchestia deshayesii is significantly different in both slope and elevation ($P < 0.01$) which may be attributable to the small weight range of the animals available.

4.3.2 Gill form

The gills of all species examined, with the exception of A. dorrieni, were similar in their external morphology (Fig. 4.1). Compared with other more marine amphipods, the gills of all the Talitridae are fairly robust, almost "sack-like" lamellae (Fig. 4.2). Each gill is attached by means of a short stalk to the posterior (medial) corner of the coxopoda of a thoracic leg (Fig. 4.2). A preliminary investigation of some thin gill sections showed that the branchial walls are formed by a single layer of epithelial cells, overlain by a cuticular covering. The cuticle is between 1 and 3 μm thick. The inner lumenal area of the gill is separated into a series of haemocoelic lacunae, which are perfused with haemolymph.

Figure 4.3 shows the percentage contribution to the total gill area of the gills on each peraeon segment for the seven species examined. Using the terminology of Moore and Taylor (1984) the gills are numbered according to the peraeopod of origin. Gill 2 (G2) in all but Arcitalitrus was large and strongly elongated anteriorly, resembling the letter "W" in outline: G6 is also large and elongate

FIG. 4.1 Outlines of the gills from seven talitrid species. Gills are from males of the same length (10mm); numbers indicate peraeopod of origin.

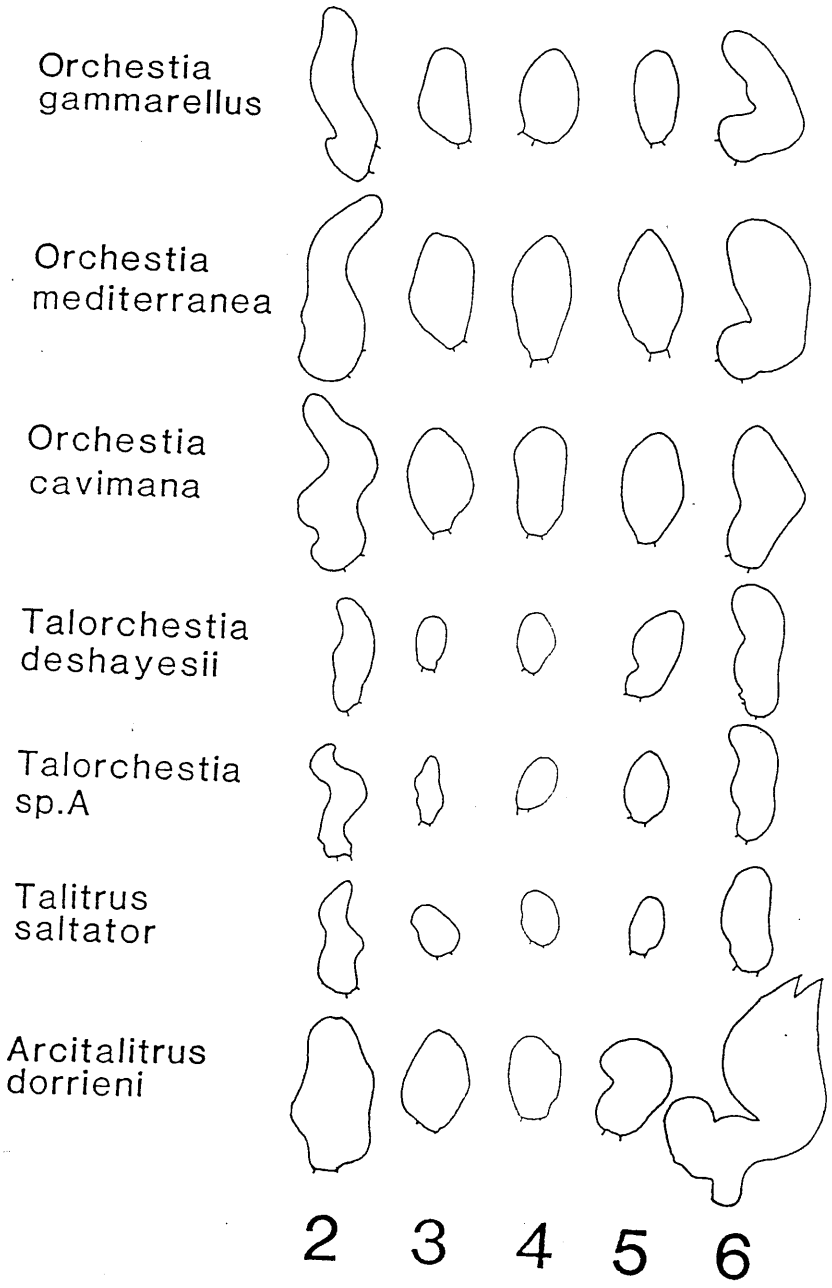
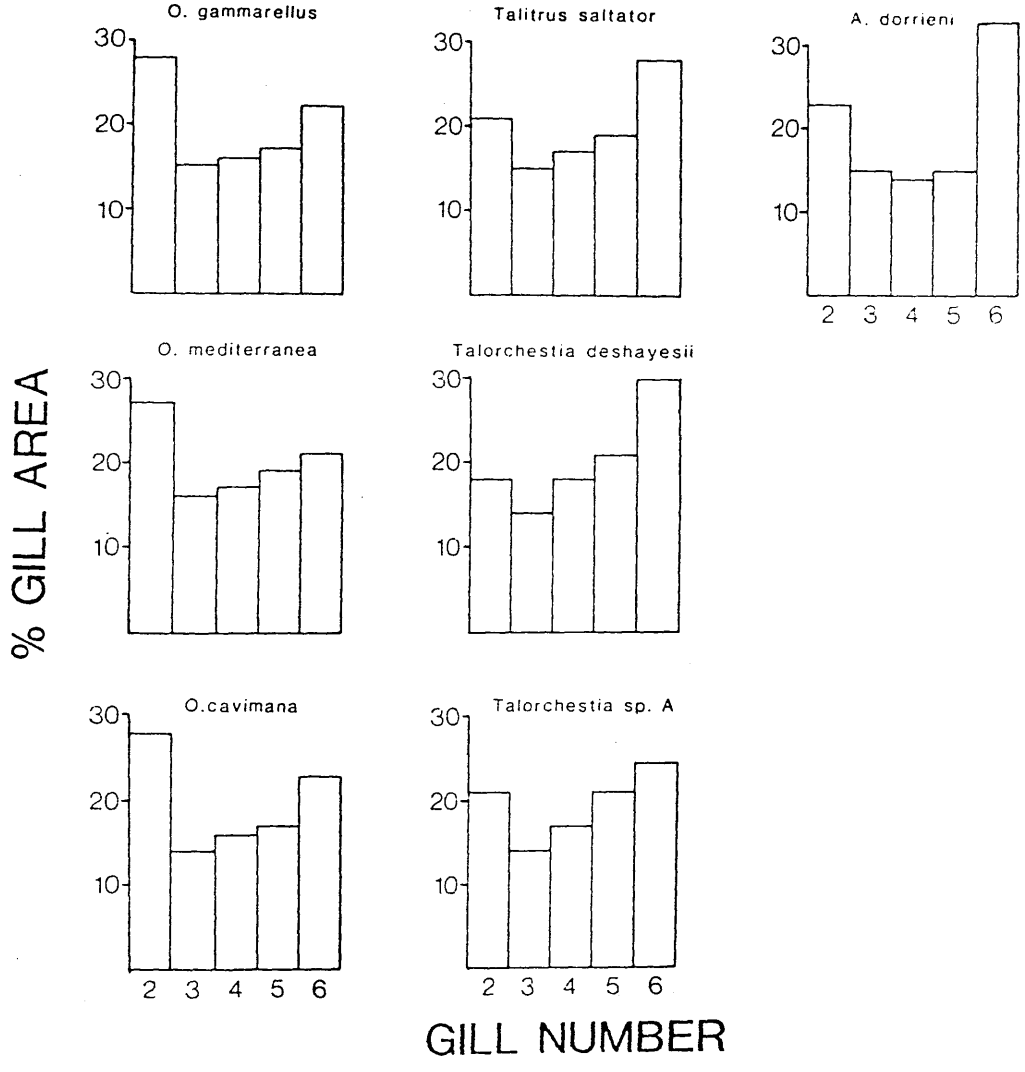

Orchestia gammarellus
Orchestia mediterranea
Orchestia cavimana
Talorchestia deshayesii
Talorchestia sp.A
Talitrus saltator
Arcitalitrus dorrieni
2
3
4
5
6

FIG. 4.2 Scanning electron micrograph of gill attached to peraeopod 4 of Orchestia gammarellus. (X 75)

CP = Coxal plate
G = Gill

FIG. 4.3 Percentage contribution to the total gill area of gills from each peraeon segment in the seven talitrid species.


CP
G


O. gammarellus
Talitrus saltator
A. dorrieni
O. mediterranea
Talorchestia deshayesii
O.cavimana
Talorchestia sp. A
30
20
10
2 3 4 5 6
% GILL AREA
GILL NUMBER

although twisting is not as pronounced as in G2 (Fig. 4.1). G 3-5 are small and simple, their relative sizes varying slightly between species. In Talorchestia, however, G.5 is of an equivalent size to G.2. G.2 is the largest of the gills in all the Orchestia spp.. This is in marked contrast to the sandhopper genera Talorchestia and Talitrus in which the greatest surface area was invested in G.6. In A. dorrieni, G.2 is fairly large with a slender L-shaped anterior extension. G.3 and 4 are small, simple and elongate while G.5 is often bilobate. As in the fossorial amphipods examined above G.6 accounts for the greatest single gill area. It is long and anseriform, being not uncommonly distally bilobate. G.7 was absent in all species examined.

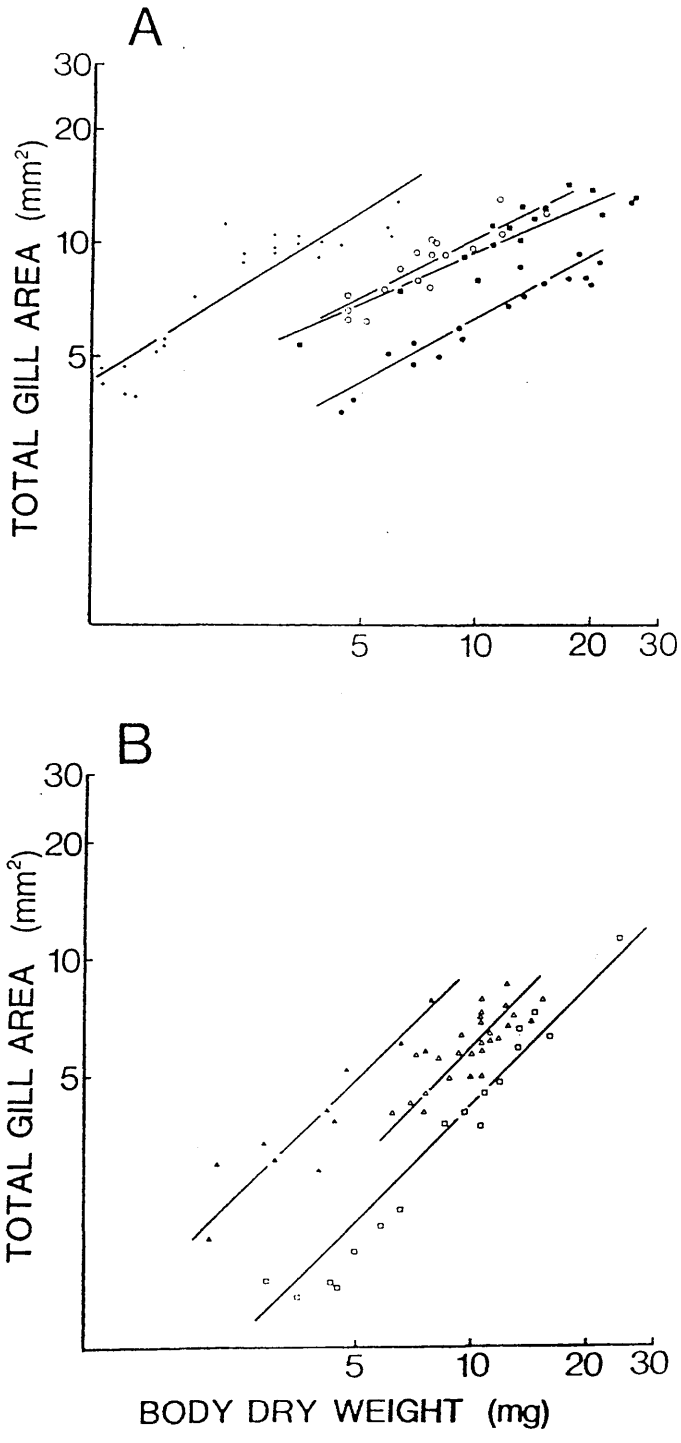
4.3.3 Gill area

Fig 4.4 shows the relationship between total gill area and dry body weight in the seven species examined. The calculated regression lines for these data are given in Table 4.2. Significant differences in either the elevations or the slopes of the regression lines were established by covariance analysis.

The total gill area of the amphipods O. mediterranea and O. cavimana, although almost identical (cf Wildish, 1979 on the close relationship between O. cavimana and O. mediterranea), were significantly greater than that of O. gammarellus of an equivalent weight (Fig. 4.4a). Covariance analysis of these data indicated that although the elevation of the log line was significantly different between these species (F_e = 104.91; d.f., 2,52; $P < 0.01$), there was no significant difference in the slope of the lines (Combined regression coefficient = 0.506). Gill areas of the large fossorial talitrids were similar to that of large O. mediterranea and O. cavimana. A significant difference in the slope of the regression line

TABLE 4.2 Regression constants for lines of best fit of total gill area (Y) on body dry weight (X) in the amphipod species investigated: equation in the form log Y = log a + b log X, values of 'a' are log numbers.

Species	Regression constants a	b	Correlation coefficient (r)	n
Orchestia gammarellus	0.252	0.549	0.94	21
Orchestia mediterranea	0.532	0.455	0.91	19
Orchestia cavimana	0.487	0.527	0.93	19
Talitrus saltator	0.368	0.994	0.97	20
Talorchestia deshayesii	-0.217	1.001	0.75	31
Talorchestia sp. A	0.010	0.977	0.89	12
Arcitalitrus dorrieni	0.641	0.920	0.92	23


A
30
20
10
5
TOTAL GILL AREA (mm²)
5
10
20
30
B
30
20
10
5
TOTAL GILL AREA (mm²)
5
10
20
30
BODY DRY WEIGHT (mg)

(eg. Talitrus saltator/O. gammarellus F_s = 26.75 ; d.f. = 1,37 ;P < 0.01) results, however, in smaller individuals of Talorchestia and Talitrus having a total gill area less than that of O. gammarellus of an equivalent weight (Fig. 4.4b). The regression lines for both Talorchestia species and Talitrus saltator were found to have the same slope (0.99) although there was a significant difference in elevation (F_e = 75.734 ; d.f.,2,59; P <0.01). Talitrus saltator had the smallest total gill area of all the species investigated. The slope of the regression line for Arcitalitrus dorrieni (Fig. 4.4a) was significantly different from Talitrus and Talorchestia spp. (F_s = 5.687; d.f.,3,78) though not from the orchestids. Arcitalitrus dorrieni had the largest total gill area of all the species investigated.

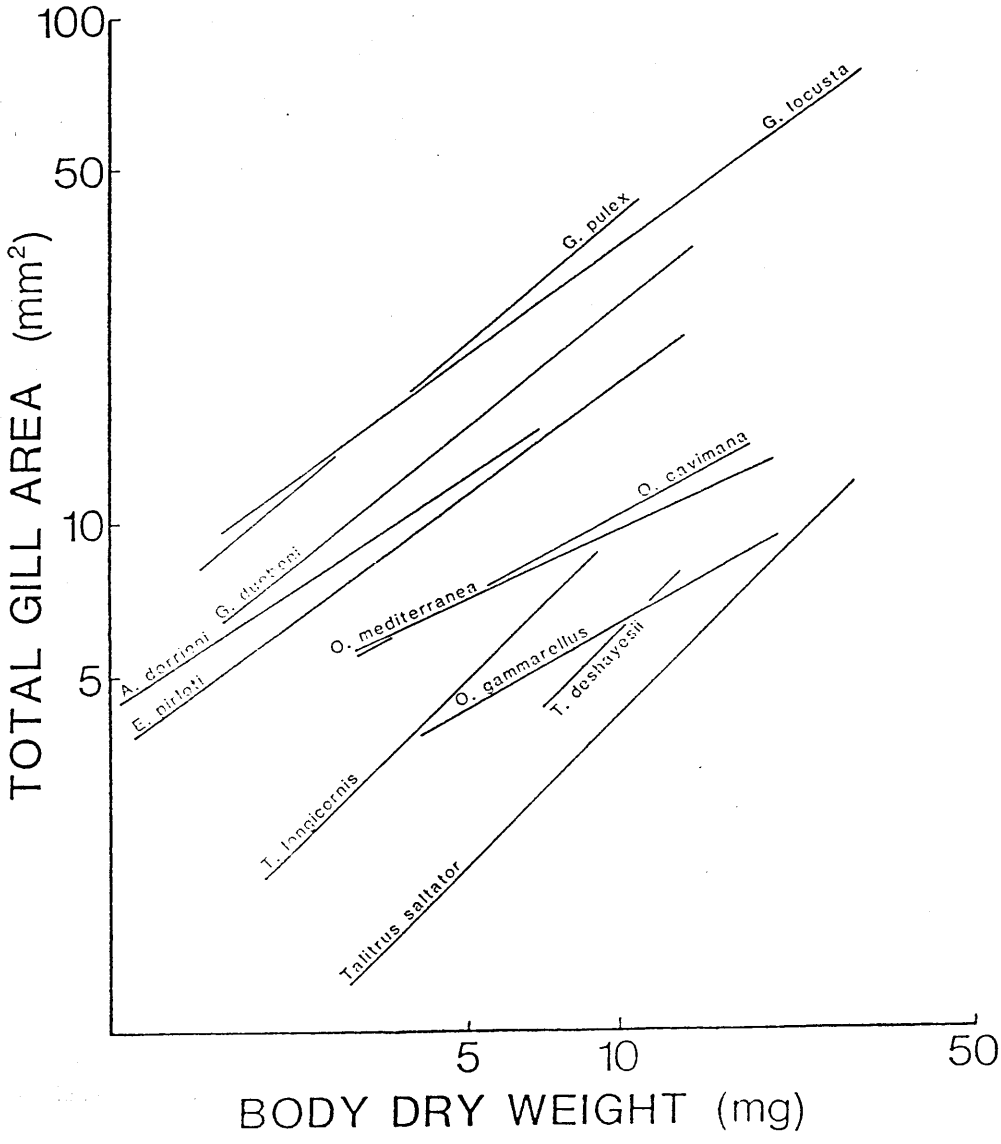
All of the talitrids examined, with the exception of Arcitalitrus, had a significantly smaller gill area than the more aquatic amphipods examined by Moore and Taylor (1984) (Fig. 4.5) The total gill area of A. dorrieni was similar to the intertidal Echinogammarus pirloti although it was smaller than that for the Gammarus species on which data exist.

4.4 DISCUSSION

4.4.1 General morphology

Delage (1881) and Nebeski (1881) were the first to comment on the morphology of talitrid gills. Nebeski compared the gills of O. gammarellus with those of Gammarus spp. and noted that whilst the structure was essentially the same in both, the gills were smaller and much more solid and stronger in Orchestia. This additional

FIG. 4.5 The calculated regression lines for the relationship between total gill surface area (mm^2) and body dry weight (mg) for the seven talitrid species, together with the regression lines calculated by Moore and Taylor (1984) for various gammarid species.


100
50
10
5
TOTAL GILL AREA (mm²)
G. locusta
G. pulex
G. duebeni
A. darrieni
E. nirlati
O. cavimana
O. mediterranea
O. gammarellus
T. deshayesii
T. longicornis
Talitrus saltator
5
10
50
BODY DRY WEIGHT (mg)

strengthening may provide support to a structure adapted to an aquatic medium, enabling it to function more efficiently in an aerial environment. This is confirmed for all the species studied, with the exception of Arcitalitrus in which the gills were very much enlarged compared with the supralittoral species, being lobate and convoluted. Nevertheless, their structure is still somewhat thicker and more irregular in outline when compared with the thin plate gills of the gammarids. Thesefeatures will inevitably affect the accuracy of gill area measurements of this species, which may be marginally underestimated.

It has been claimed that the gills are protected from drying out by a secretion from various glands (Nebeski, 1881; Bulycheva, 1957 quoted in Hurley, 1968). Although the presence of pores in the tegument of O. gammarellus has recently been reported (Sellem and Graf, 1982; Moore and Francis, 1985a), the presence of such glands as reported by Bulycheva has been regarded with some suspicion (Hurley, 1968). The present author was unable to identify any similar structure responsible for the moistening of the gill surface in the species examined.

4.4.2 Gill area distribution

Gill structure and deployment patterns are quite conservative in the talitrid species studied. The observed differences in relative gill size reported here are reasonably constant within the beachflea, sandhopper and more advanced landhoppers examined and confirm the observations by Friend (1980) and Bousfield (1982b, pers comm.). The gills attached to the second and sixth peraeopods together comprise approximately half of the total gill area. This is in full agreement with the results obtained during a study of the subtropical beachflea Chroestia lota (Marsden, 1985). In contrast, to the three Orchestia

species studied, however, in which the area of G.2 is greater than that of G.6, this pattern is reversed in both the sandhoppers and the landhopper studied. In Talitrus, Talorchestia and Arcitalitrus (cf. also Chroestia lota; Marsden, 1985) the gill with the largest surface area is the posteriormost (G.6). The posterior gills will be protected from desiccation in the quiescent animal by flexed abdomen and pleopods. In Talorchestia, since a greater percentage of the total gill area is invested in both G.5 and G.6, this may confer even greater desiccation resistance on these animals. In aquatic amphipods, since the respiratory current proceeds in an anterior to posterior direction, it is advantageous to have maximum gill area concentrated anteriorly, in the region of highest oxygen availability (Dahl, 1977). Although this may apply to Orchestia species which are often subject to various degrees of inundation at high tide, it is clearly not relevant to the fully terrestrial landhoppers.

Therefore, the retention and indeed proliferation of G.6 in A. dorrieni is difficult to explain unless there is a selective advantage in developing such a structure. Personal observations have confirmed that A. dorrieni carries a small quantity of exosomatic water in the posterior section of the ventral groove. Sporadic rhythmic beating of the extant pleopods (P 1 is vestigal) creates considerable turbulence in the exosomatic water causing G.6 to vibrate. Presumably the turbulence created at the gill surface is important as it reduces the thickness of the boundary layer that under normal circumstances would impede oxygen diffusion across the cuticular surface. It is suggested that the enlargement (and presumably the irregular shape) of the posteriormost gill in A. dorrieni, is linked with the retention of the pleopods, and that together both features provide the animal with an efficient gas exchange organ. Further studies on the functional morphology of this system are now urgently required to clarify its

role in the respiratory physiology of these animals.

When talitrids are strongly flexed, the larger and more elongate gills on P.2 and P.6 project forwards and backwards into the air, clear of the body. Together with their observed "twisting", these features would presumably allow more efficient gas exchange than would be possible at G.3-5. The W-shape of G.2 may also be related to the mechanical constraints of gnathopod deployment and stowage (cf. Moore and Taylor, 1984 on the gammarids) particularly in the strongly sexually dimorphic forms such as Orchestia. The remaining gills(G.3 - 5) are considerably smaller and less diverse in form than G.2 or G.6 (except in Talorchestia, see above). Dr. E.L. Bousfield (pers. comm.) has suggested that the reason for this reduction in size may be a mechanical one. The reduction may be a response to the need for the sternal cavity to be occupied by a rather large brood pouch (in the female) and/or to avoid abrasion of the gill surfaces by the substratum.

Dahl (1977) has suggested that the absence of G.7 in Orchestia may have occurred to prevent impedence of pleopod action. Reduction in gill number, however, is fairly common among the Crustacea, and there may be more than one reason for its occurrence (Hartnoll, 1964). Gill 7 is lacking in all ten families, including aquatic types, within the superfamily Talitroidea (Dr. E.L. Bousfield, pers. comm.).

4.4.3 Total gill area

Moore and Taylor (1984) found a reduction of total gill area in O. gammarellus as compared with other more marine species. This study confirms these results for all of the species studied with the exception of Arcitalitrus. This strengthens the argument that reduction of the gill area in amphipods can be correlated with the colonisation of the supralittoral zone (Bousfield, 1958; Moore and

Taylor, 1984; Marsden, 1985). It is generally recognised that the ability to regulate evaporative water loss represents a major factor controlling the colonisation of the higher shore levels by marine invertebrates (Newell, 1979). It is plausible, therefore, that reduction of gill area in supralittoral amphipods may have occurred to aid water conservation. There is evidence for the presence of a waxy layer in the cuticle of some talitrids (Bousfield, 1984; Moore and Francis,1985a). This may not, however, act as an effective barrier to water loss since oxygen uptake occurs across the general body surface and is not restricted to the gills (Graf and Magniez, 1969). A reduction of the total gill area as a means of restricting water loss would appear to be expedient in the face of the apparent high permeability of the general body surface. The known desiccation resistance of many of the talitrids examined in this study, however, appears to be correlated with their total gill area. Water loss is greater and desiccation tolerance lower in Arcitalitrus sylvaticus than in O. gammarellus (Lazo-Wasem, 1984). In contrast, Talitrus and Talorchestia deshayesii are more resistant to desiccation than O. gammarellus (Williamson, 1951a).

The gill area of A. dorrieni is greater than that of the supralittoral amphipods examined, but is still reduced in comparison with aquatic marine and freshwater species. The leaf litter habitat retains moisture which may render specific adaptations in Arcitalitus, considered essential in other terrestrial environments, unnecessary (see Hurley, 1959, 1968 for discussion). In organisms which are not subject to intense desiccation stress, the metabolic energy requirements may be met by aerial gas exchange through relatively unmodified gill structures (Newell,1979). Bousfield (pers. comm. in Moore and Taylor, 1984) believes that the functional significance of

the enlarged gills in the rain forest species can be attributed to their importance in ion uptake. If this is so, it is difficult to explain why a parallel situation is not found in the fresh-water talitrid Orchestia cavimana. The answer may lie in the fact that O. cavimana is restricted to western European regions where significant Na^+ and Cl^- ions are present in the rain and soil, and in fresh water runoff from hardwater soils while many landhoppers occur in humic ion-poor tropical rain forests where need for osmoregulatory surfaces is presumably much higher (Dr. E.L. Bousfield, pers. comm.).

It has also been suggested that the large gills of the fully terrestrial species have been retained to maintain oxygen at a necessary level without the aid of a respiratory current (Dahl, 1977). Lack of oxygen is, however, rarely a problem in the aerial environment (Little, 1983). Certainly oxygen consumption in O. gammarellus is unaffected by gill excision (chapter 3). Duncan (quoted in Hurley, 1968) has found, that up to 60% of total gas exchange takes place across the gills in terrestrial hoppers. It is therefore difficult to assess the validity of relating oxygen consumption rates to gill area in amphipods (Marsden, 1985) or other crustacean groups (Ambore and Venkatachari, 1978; Babula et al., 1978) without sufficient knowledge of extrabranchial routes of exchange, and the permeability of the gill epithelium often shows obvious division into osmotic and respiratory tissue, the relative proportions of which will alter the blood-gas diffusion ability of the gill. Allowance for such a partition must be made if valid interspecific comparisons of functional gill area are to be made. The thickness of the gill cuticle in O. gammarellus is similar to that found in the more marine Gammarus (Dahl, 1977), which in the case of the latter may be ten times less than the average cuticular thickness (Milne and Ellis, 1973). Such structural modification has the effect of minimising the diffusion

distance and so facilitates diffusional exchange of dissolved gases across the branchial surface. Therefore, the reduction of total gill area may be an important limiting factor in both oxygen uptake and transport in the haemolymph of these animals (see chapter 7 for discussion).

Although lack of oxygen is rarely a problem in air, air breathing animals are faced with greater difficulties in the removal of carbon dioxide (O'Mahoney and Full, 1984). There is, however, little evidence to suggest that the larger gill area in A. dorrieni is related to the problem of CO_2 elimination, since Talitrus saltator (a sandhopper which is known to undertake extensive nocturnal migrations (Bregazzi and Naylor, 1972) and is presumably prone to CO_2 accumulation at this time) has the smallest total gill area per unit weight of the animals presently examined.

The present findings support the view that there is a good semi-phyletic contrast between beachfleas and sandhoppers (Bousfield, 1984). The two groups are clearly distinguished by the characteristic slope of their gill area/dry body weight regression plots. Also the marked difference in gill area of Talitrus (also Talorchestia) as compared with Arcitalitrus highlights the morphological remoteness of sandhoppers from landhoppers. This quantitative assessment of gill patterns further strengthens the contention of Bousfield (1984) of the need to remove all known landhopper species from the genus Talitrus. The evolutionary and physiological significance of these findings will be discussed in the last chapter.

CHAPTER 5

THE OXYGEN TRANSPORTING PROPERTIES OF THE HAEMOLYMPH OF *ORCHESTIA GAMMARELLUS* AND *O. MEDITERRANEA**

5.1 INTRODUCTION

There is an extensive literature on the influence of various factors such as pH, temperature, presence of divalent ions and lactate accumulation on the oxygen transporting properties of the blood of decapod Crustacea (Hogben, 1926; Redfield, 1934; Redmond, 1955; Larimer and Riggs, 1963; Truchot, 1975; Mangum and Towle, 1977; Brower et al.,1978; Johnston and Becker, 1981; Mauro and Mangum, 1982; Mason et al., 1983; Bridges et al., 1984; see also Mangum, 1980, 1983a; Truchot, 1983 for reviews) but our knowledge of other groups is severely limited. Among the isopods, for example, there have been a few studies concerned mainly with electrophoretic descriptions of haemocyanins (Berthet and Berthet, 1963; Wieser, 1965; Alikhan, 1971; Alikhan et al., 1974) or, more recently, with comparative studies of the absorption spectra and amino acid composition of oniscoidean isopods (Sevilla, 1978). Although some data have recently been published on the haemocyanin oxygen carrying capacity and blood pH of Mesidothea entomon, a species which occurs in the Baltic and in some Swedish lakes (Hagerman and Oksama, 1985) only the studies of Sevilla and Lagarrigue (1979) on an ecological series of woodlice and of Jokumsen et al (1981) on the giant Antartic isopod, Glyptonotus antarticus have provided detailed information on the role of haemocyanin in blood oxygen transport.

Whilst information on the isopods is limited, there have been, to

* Much of this chapter is embodied in a paper by Taylor, A.C. and J.I. Spicer (1986) The oxygen transporting properties of the haemolymph of two semi-terrestrial amphipods, Orchestia gammarellus (Pallas) and O. mediterranea (Costa).J. exp. mar. Biol. Ecol., 97, 135-150.

my knowledge, no studies on the oxygen transporting properties of haemolymph from amphipod Crustacea. The existence of haemocyanin in the blood of amphipods was questioned by Rawlinson (1937) but was later confirmed by Manwell and Baker (1963) and Berthet and Berthet (1963). The only other information of any sort that is available consists of electrophoretic descriptions of blood proteins (in particular the vitellogenic protein: see Charniaux-Cotton and Payen, 1985 for review) and comparative data on haemocyanin concentrations (Berthet and Berthet, 1963; Wieser, 1965; Alikhan et al., 1974). The lack of information on the properties of the haemocyanin in groups such as the isopods and amphipods is probably attributable to the difficulties of obtaining blood samples from such small animals and to the volumes of blood needed for some of the earlier techniques used for obtaining in vitro oxygen dissociation curves. Information on the respiratory properties of the haemocyanin in groups other than the decapods is urgently required, however, if we are to obtain a better understanding of the functional significance of interspecific variations in these properties within the Crustacea as a whole.

This chapter sets out to examine some aspects of the oxygen transporting properties of the blood in two semi- terrestrial amphipods, Orchestia gammarellus and O. mediterranea. They inhabit two distinct zones high on the shore of the Isle of Cumbrae (see chapter 2 for full details). Here, they are very often subject to different degrees of inundation, and considerable fresh water influence and temperature fluctuation (Rankin and Davenport, 1981). The oxygen binding properties of haemocyanin are usually sensitive to temperature shifts (Truchot, 1973; Mauro, 1978; Mangum and Lykkeboe, 1979) and changes in the ionic environment (Wheatly and McMahon, 1981; Mason et al., 1983), but the nature of the responses are very often

different (Weiland and Mangum, 1975). It is hoped that this study will serve as a preliminary investigation into the various ways in which these factors and their interactions influence the role of haemocyanin as an oxygen carrier *in vivo*.

Secondly, previous work carried out on some selected decapods (Redmond, 1968; Bliss, 1968; Young, 1972) and on some oniscoidean isopods (Sevilla and Lagarrigue, 1979) suggests that there is a general trend towards a decrease in the oxygen affinity of the pigment associated with adaptation to the land habitat. Sevilla and Lagarrigue (1979) suggest that this evolution may be related to a morphological and functional specialisation of the respiratory organs. On the other hand, Mangum (1982) states that when animals from the same geographical location are compared, this association of lower oxygen affinity with the terrestrial habit is reversed when the crustaceans are viewed as a whole: in fact, within the decapods alone there is no clear relationship between oxygenation properties and the nature of the gas exchange system.

Moore and Taylor (1984) in their study of the gills of amphipods in an ecological series, have noted a reduction in the total gill area of *O. gammarellus* compared with other more marine amphipods (see also chapter 4). This they attributed to waterloss limitation and concluded that the gill area reduction is related to the colonisation of the land. This is in full agreement with similar studies on the decapods (Ayers, 1938; Gray, 1957). Finally they note that, "...the full significance of our morphological findings....will only become apparent once comparative data on respiration rates, blood haemocyanin content and oxygen affinity become available for the species studied." Information regarding the blood gas relations of *O. gammarellus* and *O. mediterranea* may throw more light on the relationship between pigment affinity and terrestrial adaptation in the Crustacea.

5.2 MATERIALS AND METHODS

5.2.1 Animal material

Orchestia gammarellus and O. mediterranea were collected from M.H.W.S. and M.H.W.N. at Farland Bight (O.S. Grid ref. NS 17 324412), Ballochmartin Bay (O.S. Grid ref. NS 17 318437) and Fintray Bay (O.S. Grid ref. NS 17 331441) on Great Cumbrae Island in the Firth of Clyde. The animals were transported back to the laboratory in plastic bags containing some of the substratum in which they were found.

5.2.2 Blood samples

All blood samples were taken from animals within 12 hours of capture. Only animals corresponding to the intermoult stage C (Charniaux-Cotton, 1957) were used in this study, since it was observed that in newly moulted animals, the haemocyanin content of the blood was very low (see below). Blood was taken using a fine-tipped Hamilton glass syringe to puncture a small dorsal hole between the second and third thoracic somites. Great care was exercised to avoid damage to any of the fat bodies present beneath the cuticle and to ensure that the animals were disturbed as little as possible prior to blood sampling in an attempt to minimise the possible accumulation of lactate in the blood which is now known to affect the oxygen transporting properties of crustacean haemocyanins (Truchot, 1980; Booth et al., 1982; Mangum, 1983b; Bridges et al., 1984; Morris et al., 1985).

The blood was drawn up slowly into the syringe and then transferred to a 1.5 ml Eppendorf tube stored on ice. Due to the small size of even the largest individuals (fresh weight range 40 - 150 mg), it was normally possible to obtain only 5 - 15 µl of blood from an

individual animal. It was therefore necessary to pool blood from many animals to obtain sufficient blood for subsequent analysis. The pooled blood sample was then mixed thoroughly, centrifuged at 10,000 g for 10 minutes to remove any cells and coagulated proteins and stored deep frozen.

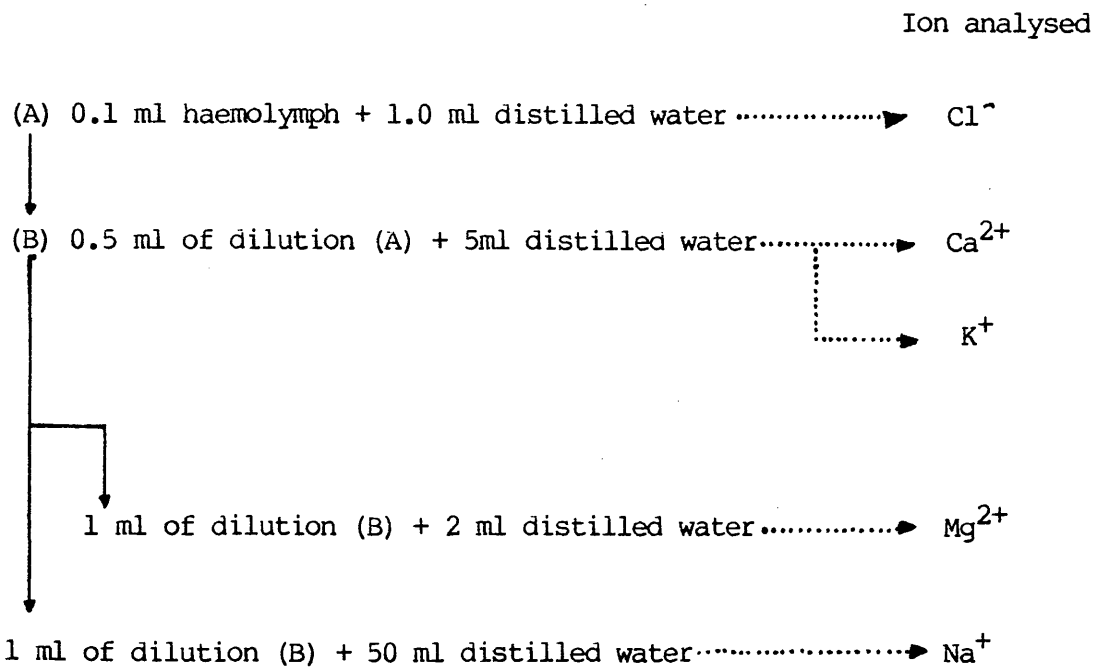
5.2.3 Analysis of haemolymph ionic, protein and lactate content

The concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} ions in the blood was determined using an atomic absorption spectrophotometer (Pye Unicam SP90). The dilution scheme used is outlined in Fig. 5.1. The concentration of Cl^- ions was determined using a chloride titrator (E.E.L. 920). The concentrations of L-lactate in the pooled blood samples were determined spectrophotometrically using the method of Gutman and Wahlefeld (1974) with the modifications used by Graham et al. (1983). 50 ul of blood was deproteinised by the addition of 0.3M perchloric acid and centrifuged at 10,000 g for 15 minutes. The resultant supernatant was neutralised with $KHCO_3$, and 50 µl were removed to an Eppendorf tube (1.5 ml) containing 1000 µl of Hydrazine/ Glycine buffer and 50 µl of NAD^+ (40 mM). The enzyme lactate dehydrogenase (5 mM) was then added. The resultant mixture was incubated in a waterbath at 37^0C for one hour before the absorbance was read on a Pye-Unicam PU 8600 spectrophotometer at 340 nm. Lactate concentration in the blood sample was calculated using the extinction coefficient (E_{1cm}) for lactate of 6.22 µmoles.ml^{-1}.

Measurements were also made of the total protein concentration of blood samples from 37 individual animals and of the pooled blood samples using the Coomassie Blue method (Read and Northcroft, 1981). The blood samples (5 - 10 µl) were diluted with 100 -200 µl of distilled water and 50 µl of the resulting solution was transferred to a spectrophotometric cell (volume = 1.0 ml: path length = 1cm)

FIG. 5.1 Dilution scheme used for measuring the concentration of the major ions in the haemolymph of Orchestia gammarellus and O. mediterranea.

DILUTION SCHEME



together with 950 µl of Coomassie reagent. The solution was rapidly mixed and left for 10 minutes at room temperature before the extinction at 595 nm was measured on a Pye-Unicam PU 8600 spectrophotometer.

5.2.4 Blood pH and oxygen content measurements

The pH of the post-branchial blood was determined in 34 animals as described above but instead of drawing the blood sample into a syringe, the blood was drawn directly into the capilliary pH electrode of a Radiometer BMS2 which was connected to a PHM73 pH meter (Radiometer, Copenhagen). As the electrode capacity is approximately 30 µl and the maximum blood volume obtained from the largest talitrid was rarely in excess of 15 µl, the extra volume was made up with KCl electrolyte. If any obvious mixing took place at the blood/KCl interface, the result was discounted. The reliability of this method was verified using both small and large samples of haemolymph, freshly extracted from large male shore crabs (Carcinus maenas).

The BMS2, thermostatted at 10^0C, was also used to tonometer blood samples with air and the total oxygen content of the blood (Co_2) was determined in 10 µl samples using the method of Tucker (1967) as modified by Bridges et al. (1979). The oxygen carrying capacity of the haemocyanin ($C_{HCY}O_2$) was then calculated by subtraction of the physically dissolved fraction (see below).

5.2.5 Oxygen dissociation curves

In vitro oxygen dissociation curves for the blood of both species were constructed at three temperatures, viz. 10, 15 and 18^0C. The blood was tonometered in a Radiometer BMS2 at the appropriate temperature with gas mixtures supplied by precision gas mixing pumps (Wosthoff, Bochum, F.R.G.). After 20 - 30 minutes equilibration, the oxygen content of a 10 µl sample of blood was determined using the

method of Bridges et al. (1979) in which potassium cyanide is used to release O_2 bound to the haemocyanin. The total oxygen content of the blood sample was calculated using the following equation:-

$$\text{Total oxygen content } (C_{HCY}O_2 = Po_2 / 760 \times aCN^- \times Ch.V. \times 100/S.V.$$

where aCN^- = Solubility coefficient for oxygen in KCN^- which at 30^0C = 0.0261

Ch.V = Chamber Volume (300 µl)

S.V. = Sample Volume (µl)

$$Po_2 = Po_2 (KCN^- + Blood) - Po_2 (KCN^-) \times (Ch.V - S.V.)/Ch.V$$

The haemocyanin-bound oxygen content of the blood sample was calculated from the following equation:-

$$\text{Bound } O_2 = \text{Total } O_2 \text{ content} - \text{physically dissolved } O_2$$

(Units = mls of oxygen per 100 ml of blood)

The physically dissolved oxygen in a physiological saline having an ionic composition approximate to the blood sample was measured at various partial pressures of oxygen using the Tucker cell as outlined above. The values obtained were taken to be equivalent to the amount of oxygen physically dissolved in the blood at the appropriate partial pressures.

Values of $C_{HCY}O_2$ calculated for blood equilibrated to a range of oxygen tensions were calculated and expressed as percentage saturation. The P_{50} and cooperativity (n_{50}) of the blood were estimated from the regression lines for the saturation values (between 25 -75 % saturation) according to the Hill equation. Blood pH was varied by altering the partial pressure of CO_2 in the gas mixtures and the pH of the blood near the P_{50} was measured using the capillary pH

electrode of a Radiometer BMS2.

The effect of temperature on the oxygen transporting properties of the blood was investigated by calculating the change in enthalpy (ΔH) accompanying oxygenation of haemocyanin with respect to the temperature change at a constant pH (7.8) using the equation

$$\Delta H = (-2.303 \times R \times \log P_{50}) / (1/T1 - 1/T2) \text{ kJ.mole}^{-1}$$

where R is the gas constant and T the absolute temperature.

5.2.6 Further investigations on O. gammarellus

During preliminary studies on the blood of these amphipods it was observed, in O.gammarellus, that the oxygen transporting properties of the blood varied somewhat according to the exact location on the shore from which the animals were collected. Further studies were therefore carried out to examine this in more detail. Orchestia gammarellus were collected from two locations on the shore: the first group from beneath rocks and shore debris lying on the grass verge bordering the beach well above M.H.W.S. (AHW animals); the second group from beneath rocks and stones in the zone occupied by the alga, Pelvetia canaliculata (L.) Dicne et Thur. at the lower limit of the amphipods' distribution on the shore (BHW animals). The animals were returned to the laboratory and blood samples taken immediately using the procedures described above.

The concentrations of the major ions in the blood were determined and in vitro dissociation curves constructed using the techniques described previously. Further experiments were carried out to investigate the effect of differences in the ionic concentration of the blood on the oxygen transporting properties of the haemocyanin. During these experiments, samples of pooled blood (800 - 1500 µl) from BHW animals were dialysed against different physiological saline solutions. Blood from BHW animals was dialysed against a saline having

an ionic composition identical to that of AHW animals. In a second experiment, blood from BHW animals was dialysed against another saline which had an ionic composition identical to the first except that the concentration of calcium ions was increased to the same concentration as in the blood of BHW animals. A control experiment was also performed in which blood from BHW animals was dialysed against a physiological saline the ionic composition of which was identical to that of the blood of BHW animals. The blood samples were dialysed for 24 hours at 5^0C in 500 ml of saline solution. The solution was gently stirred during the dialysis period and was also covered to minimise evaporative water loss. Following dialysis, dissociation curves for each of the blood samples were constructed using the above procedure.

5.3 RESULTS

5.3.1 Ionic composition of the blood

Mean values for the concentration of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- ions in pooled blood samples of O. gammarellus and O. mediterranea are given in Table 5.1. There were only small differences in concentration between these two species, none of which, with the exception of the Cl^- and Na^+ ion concentrations, were significantly different. The concentration of Na^+ and Cl^- ions in the blood of O. mediterranea was somewhat lower than in O. gammarellus.

The values in Table 5.1 are for blood samples obtained from animals collected from those areas of the shore at which they were most abundant and have therefore been taken to represent the typical concentrations of these ions in the blood of these two species. Orchestia gammarellus were occasionally found, however, in small groups under large rocks or shore debris much higher in the

TAB. 5.1 The concentration of the major ions and of protein in the blood of Orchestia gammarellus and O. mediterranea collected from the middle of their distributions on the shore. Values are means ± S.D. of 5 and 3 pooled blood samples respectively. In vivo pH values for the blood of both species are also given (n = 10-15).

	O. gammarellus	O. mediterranea
Na^+ mmol.l^{-1}	548.8 ± 46.8	500.3 ± 26.0
K^+	17.9 ± 2.7	15.6 + 1.2
Ca^{2+}	15.5 ± 3.2	13.4 ± 2.5
Mg^{2+}	23.9 ± 4.0	21.8 ± 2.0
Cl^-	558.2 ± 59.1	492.0 ± 3.1
Total protein mg.ml^{-1}	22.1 ± 1.5	23.0 ± 1.7
pH	7.83 ± 0.16	7.87 ± 0.10

supralittoral zone (AHW animals) (see also chapter 5). Although they were not abundant at these locations, blood samples were obtained from these animals to compare ionic composition with that of pooled samples from the lower shore BHW animals The results of these analyses are presented in Table 5.2.

The concentrations of the major ions in the blood of BHW animals (Table 5.2) were almost identical to those in the blood of animals collected from the middle of their occupied zone on the shore (Table 5.1). The concentrations of these ions, however, were very different from those in blood samples from AHW animals. The concentrations of Na^+, Cl^- and Ca^{2+} ions in particular were very much lower in the blood of AHW animals. The reduction in the concentrations of Ca^{2+} and Mg^{2+} ions in the blood of AHW animals was especially interesting since these ions are known to play an important role in modulating the respiratory properties of the haemocyanin (Truchot, 1973, 1975; Weiland and Mangum, 1975; Mangum and Towle, 1977; Brower et al., 1978; Mason et al., 1983). The results of investigations of the effects of the differences in ionic composition of the blood on haemocyanin oxygen transport are given below.

5.3.2 Blood protein and oxygen carrying capacity

Values for in vivo pH, protein and haemocyanin oxygen carrying capacity ($C_{HCY}O_2$) of the blood of O. gammarellus and O. mediterranea are also shown in Table 5.1. No significant differences were found between these values in the two species (t-test, P=0.05). There was a slight difference in the protein concentration of the blood of O. gammarellus collected from well above the High Water Mark (AHW animals) and that of animals collected from the middle of their distribution on the shore but this difference was not statistically

TAB. 5.2 The concentrations of the major ions and of total protein in the blood of Orchestia gammarellus collected from above MHWN (AHW animals) and from the lower limit of their distribution on the shore (BHW animals). Values are means ± S.D. (n = 10) for pooled blood samples. In vivo pH values for the blood of AHW animals are also shown.

	BHW animals	AHW animals
Na^+ mmol.l^{-1}	550.7 ± 28.2	347.0 ± 17.8
K^+	18.4 ± 1.5	14.1 ± 0.8
Ca^{2+}	17.1 ± 3.4	9.2 ± 2.2
Mg_{2+}	24.3 ± 2.9	19.1 ± 3.3
Cl^-	567.1 ± 31.7	279.3 ± 14.2
Total protein mg.ml^{-1}	22.4 ± 2.9	27.1 ± 6.3
pH	7.83 ± 0.16	7.63 ± 0.12

significant (Table 5.1). It was found, however, that the pH of the blood of AHW animals was significantly lower than that of BHW animals ($P<0.0$ (Table 5.2).

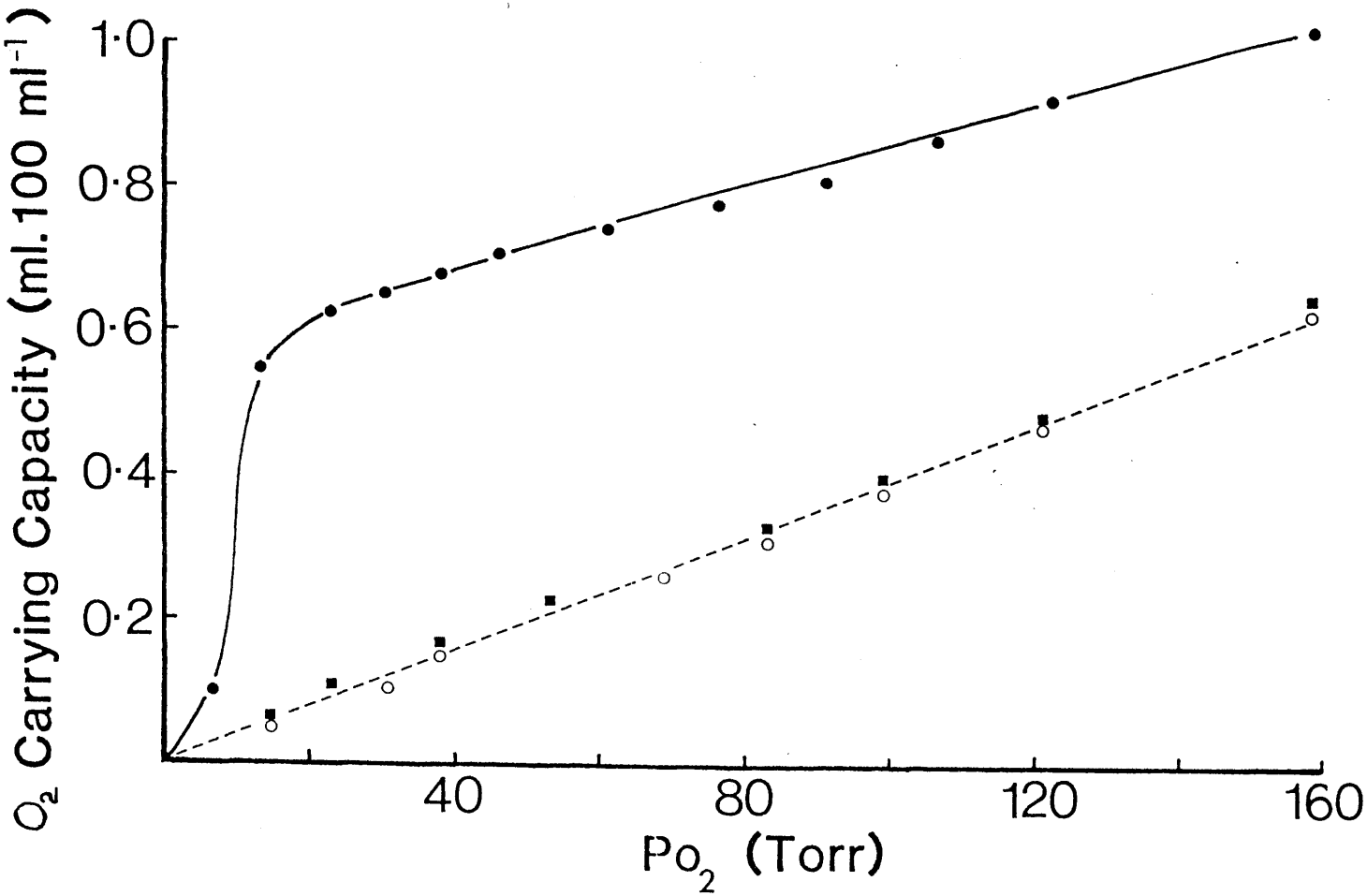
The oxygen carrying capacity of the haemocyanin ($C_{HCY}O_2$) of both species was quite low, 0.74 ± 0.20 (n = 40) and 0.69 ± 0.16 (n = 26) ml.100 ml^{-1} (0.33 $\mu mol.ml^{-1}$ and 0.31 $\mu mol.ml^{-1}$) in O. gammarellus and O. mediterranea respectively. There was no significant difference between these values (t-test, P=0.05). Calculations of the oxygen carrying capacity from the values for protein concentrations of the blood were worked out by assuming that haemocyanin represented approximately 80% of the total protein content of the blood (Wieser, 1965) and that the oxygen binding site in this species has a molecular weight of 75,000 daltons in common with many other crustaceans (Mangum, 1983a). The calculated values thus obtained were in good agreement with the measured values.

5.3.3 Lack of pigment in O. mediterranea

During this study it was occasionally found that the blood of O. mediterranea was almost completely lacking in haemocyanin. The protein concentration of such blood was extremely low (0.05 $mg.ml^{-1}$) and oxygen dissociation curves constructed for this blood were not sigmoidal (Fig. 5.2). When the blood was equilibrated against gas mixtures containing different partial pressures of oxygen and the total oxygen content of the blood calculated, a simple linear relationship was obtained which was almost identical to that obtained using either seawater or a physiological saline solution. Blood samples taken about two months later (May) from the same population of animals had normal oxygen carrying capacities and gave typically sigmoidal dissociation curves.

FIG. 5.2 The mean oxygen carrying capacity of the blood of Orchestia gammarellus and O. mediterranea was approximately 1.2 ml.100 ml^{-1}. It was found, however, that in many newly moulted O. mediterranea (■) the blood contained very little haemocyanin with the result that the oxygen carrying capacity of the blood was very low and differed little from that of sea water (○).

(•) Haemolymph from Intermoult O. mediterranea


O_2 Carrying Capacity (ml.100 ml^{-1})
1·0
0·8
0·6
0·4
0·2
40
80
120
160
Po_2 (Torr)

5.3.4 Oxygen dissociation curves

Oxygen dissociation curves for the native blood of *O. gammarellus* at 10^0C are shown in Fig. 5.3. There was very little difference in either the oxygen affinity or the size of the Bohr effect between the two species. The blood of *O. gammarellus* and *O.mediterranea* had a moderately high oxygen affinity. The P_{50} measured at the *in vivo* pH (pH = 7.8) was 9.7 and 10.3 Torr in *O. gammarellus* and *O. mediterranea* respectively. The relationships between P_{50} and pH of the blood of *O. gammarellus* and *O. mediterranea* are shown in Fig. 5.4. The blood of both species demonstrated a normal Bohr effect. The Bohr factor ($\Delta \log P_{50} / \Delta pH$) at 10^0C had a value of -0.84 in *O. gammarellus* and -0.81 in *O. mediterranea*. Covariance analysis of these data demonstrated that there was no significant difference in the size of the Bohr factor between the two species. In *O. gammarellus* and *O. mediterranea*, the oxygen affinity of the blood remained almost independent of temperature in the range 10 - 18^0C (Fig. 5.4). At pH = 7.8 the calculated values for ΔH between 10 - 18^0C were -11.7 and -21.3 kJ.mole^{-1} in *O. gammarellus* and *O. mediterranea* respectively.

The cooperativity (n_{50}) of the haemocyanin in both *O.gammarellus* and *O. mediterranea* was quite low and was largely independent of pH within the pH range 7.2 - 8.2. Cooperativity also appeared to be unaffected by temperature within the range 10 - 18^0C. The mean values for n_{50} in *O. gammarellus* and in *O. mediterranea* were 3.00 $\pm$ 0.28 and 2.94 $\pm$ 0.42 respectively.

5.3.5 Further studies on *O. gammarellus*

Since the blood obtained from *O. gammarellus* living high on the shore (AHW animals) had an ionic composition very different from that of animals collected from the lower limits of distribution, further studies were carried out to compare the respiratory properties of the

FIG. 5.3 Oxygen dissociation curves for the blood of Orchestia gammarellus. The curves were constructed at 10^0C and the pH of the blood was altered by adjusting the percentage of CO_2 in the gas mixtures with which the blood was equilibrated.

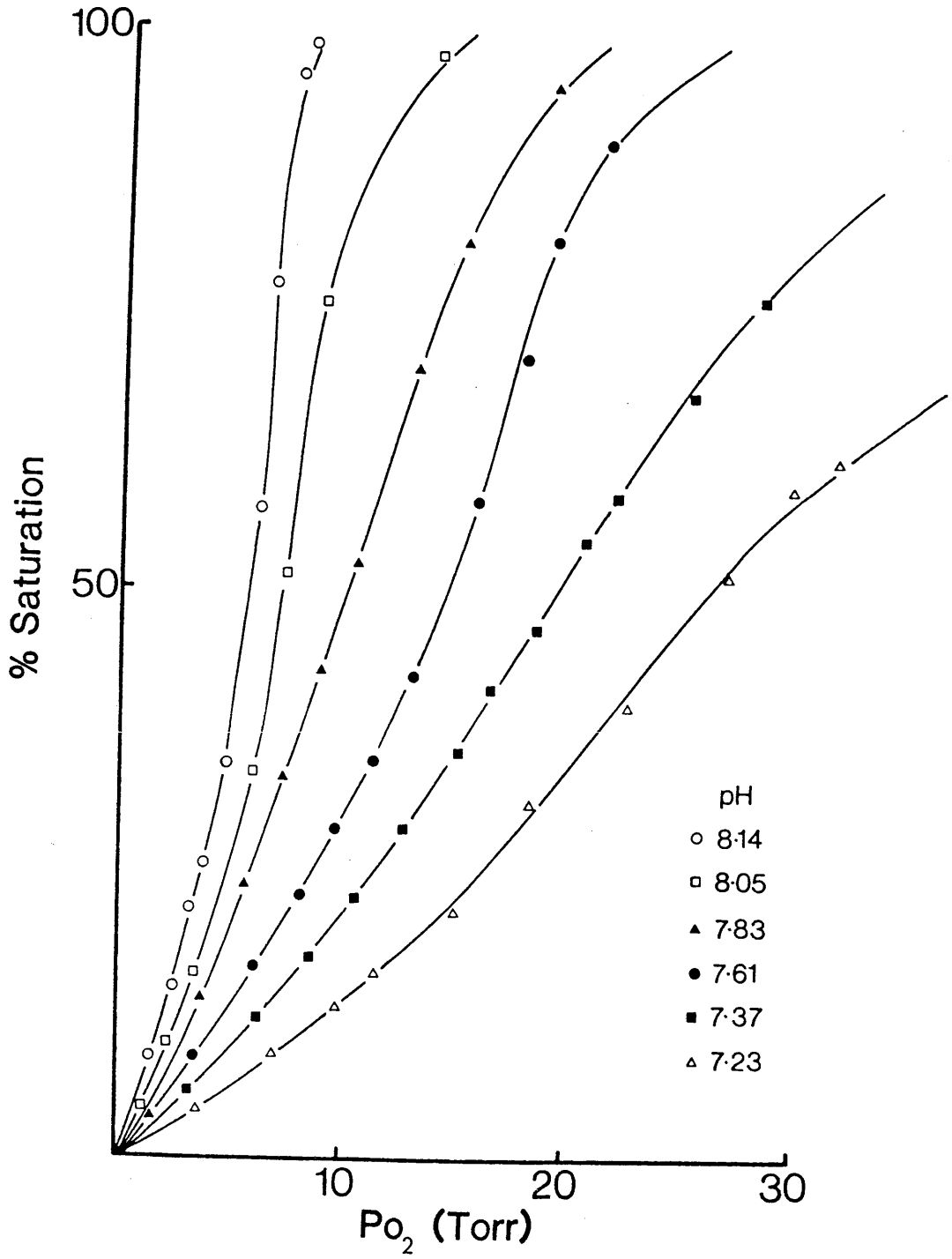
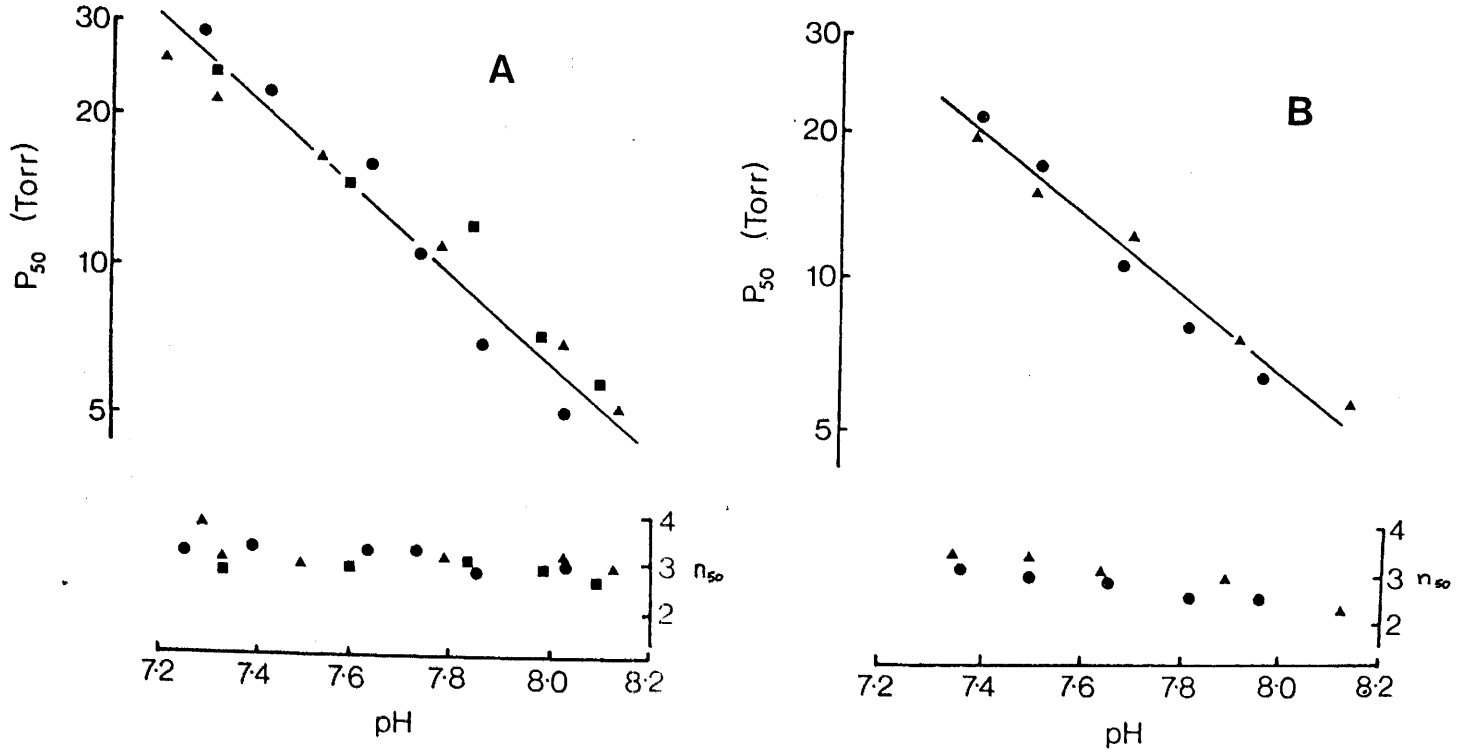

100
50
% Saturation
10
20
30
Po2 (Torr)
pH
8·14
8·05
7·83
7·61
7·37
7·23

FIG. 5.4 The effect of temperature on the relationship between P_{50} and pH for the blood of A) Orchestia gammarellus and B) O. mediterranea. Values of P_{50} were obtained from oxygen dissociation curves constructed at 10 (▲), 15 (●) and 18 (■) ^{0}C. Values of n_{50} obtained from the same curves are also given.


A
B
30
20
10
5
P_{50} (Torr)
4
3
2
n_{50}
7·2
7·4
7·6
7·8
8·0
8·2
pH

haemocyanin in these two groups of animals.

The lactate concentrations in the pooled blood samples from the two groups of animals were low (0.28 and 0.37 $mmol.l^{-1}$ in AHW and BHW animals respectively). The relationships between P_{50} and pH in both AHW and BHW animals are shown in Figure 5.5 together with values for cooperativity (n_{50}). It is clear from these data that the haemocyanin of AHW animals had a much lower oxygen affinity than that of BHW animals. At a constant pH (7.8), the haemocyanin of AHW animals had a P_{50} of 14.5 Torr whereas in BHW animals the P_{50} was 9.9 Torr. The *in vivo* pH of the blood of AHW animals was, however, significantly lower (pH = 7.6) than that of BHW animals (pH = 7.8). At the *in vivo* pH, the oxygen affinity of the haemocyanin was even lower (P_{50} = 20.5 Torr). Covariance analysis of these data showed that, although there was no significant difference in the slopes of the regression lines fitted to the data, the elevations of the lines were significantly different ($P < 0.05$). Thus, although the oxygen affinity of the respiratory pigment did differ between the two groups of animals, there was no significant difference in the magnitude of the Bohr effect. The mean Bohr value, calculated from the covariance analysis was -0.84 which was very similar to the value obtained for the blood of *O. gammarellus* collected from the middle of their littoral distribution.

Experiments were also carried out to investigate to what extent the observed differences in the ionic composition of the blood were responsible for the lower oxygen affinity of the haemocyanin of AHW animals. The results of these experiments, in which blood from BHW animals was dialysed against physiological saline solutions having differing ionic compositions are presented in Fig. 5.6. When blood from BHW animals was dialysed against a physiological saline having an ionic composition identical to that of the blood of AHW animals, the

FIG. 5.5 The relationship between P_{50} and pH for the blood of Orchestia gammarellus collected from the upper (O) and lower (●) limits of their littoral distribution (AHW and BHW animals respectively). Values for n_{50} are also given. For further details, see text.

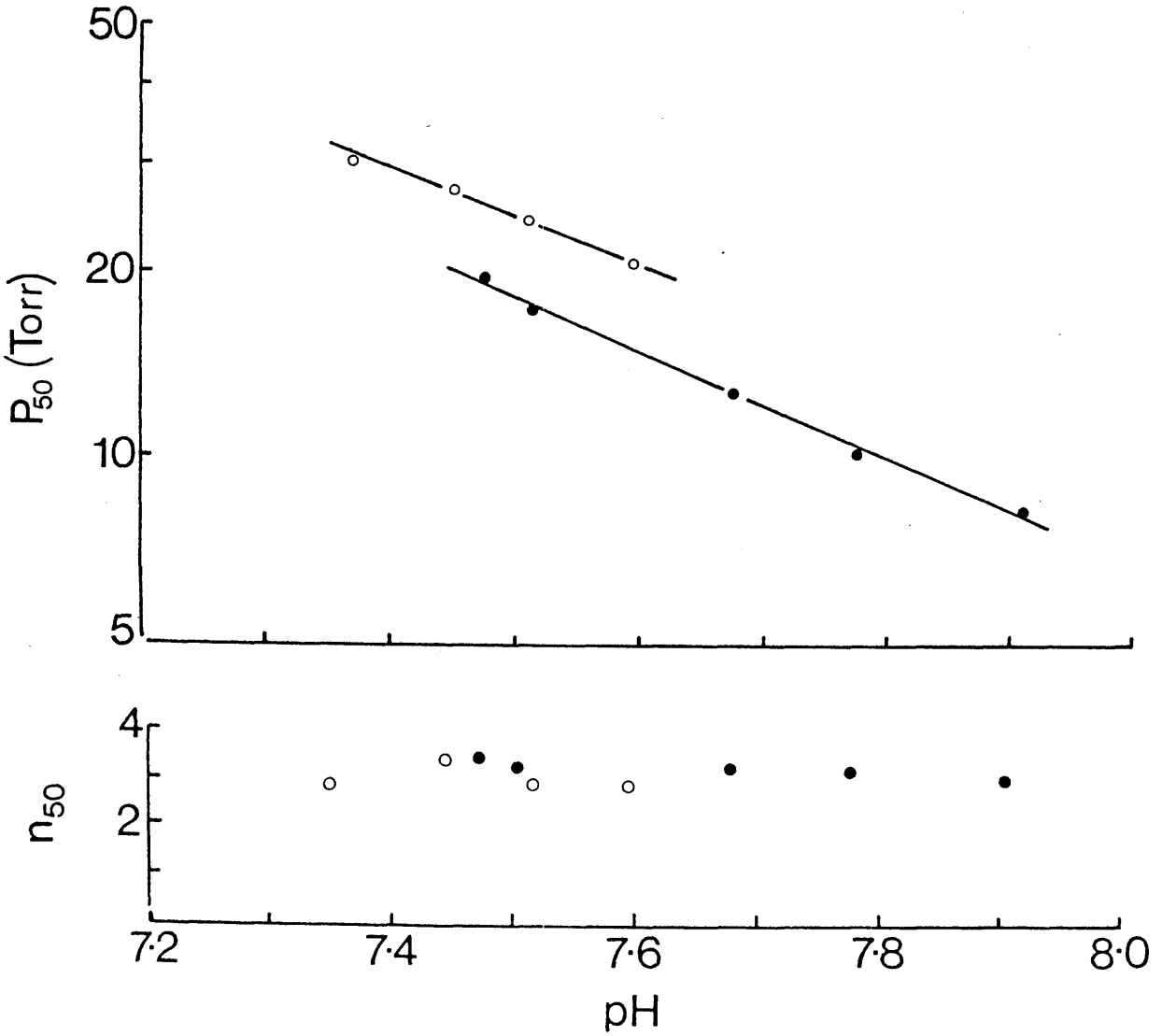
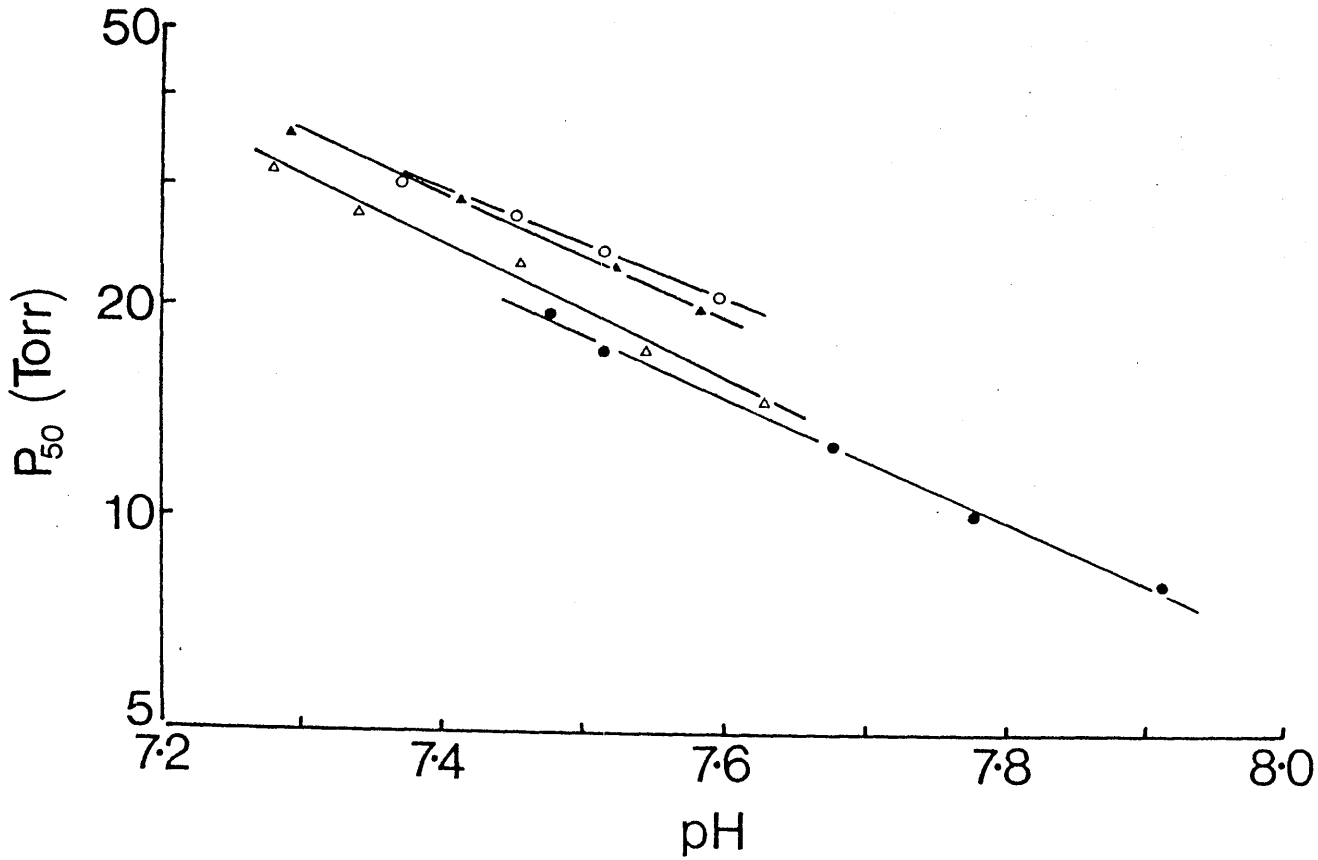

50
20
10
5
P_{50} (Torr)
4
2
n_{50}
7·2
7·4
7·6
7·8
8·0
pH

FIG. 5.6 The relationship between P_{50} and pH for the blood of Orchestia gammarellus collected from the upper (○) and lower (●) limits of their littoral distribution (AHW and BHW animals respectively). Data are also presented for blood from BHW animals which had been dialysed against a physiological saline having an ionic composition identical to that of the blood of AHW animals (▲), and against another saline having an identical ionic composition except that the concentration of Ca^{2+} ions was increased to the same concentration measured in the blood of BHW animals (△). For further details, see text.


50
20
10
5
P_{50} (Torr)
7·2
7·4
7·6
7·8
8·0
pH

relationship between the P_{50} and pH was almost identical to that obtained for whole (undialysed) blood of AHW animals. Unfortunately, only a few data could be obtained from dialysed blood due to the small volume of blood available. Nevertheless, covariance analysis showed that there was no significant difference between either the slopes or the elevations of the calculated regression lines.

When blood from BHW animals was dialysed against a physiological saline based on the ionic composition of the blood of AHW animals but having a Ca^{2+} ion concentration identical to that of BHW animals, the oxygen affinity of the blood was increased. The regression line fitted to these data was not significantly different from the line fitted to the data for whole (undialysed) blood from BHW animals (covariance analysis $P = 0.05$). A control experiment in which blood from BHW animals was dialysed against a physiological saline having an ionic composition identical to that of BHW animals was carried out. The relationship between P_{50} and pH obtained for this dialysed blood did not differ significantly from that obtained for whole blood from these animals.

5.4 DISCUSSION

5.4.1 Ionic composition of *O. gammarellus* and *O. mediterranea* haemolymph

The relative proportions of ions in both *Orchestia* species was indicative of a highly developed system of ionic regulation, comparable to that of many of the decapod and isopod species examined to date. The blood magnesium concentration was less than half that found in normal sea water. This is a common feature of many aquatic and terrestrial crustaceans (Robertson, 1949; McLusky, 1968; Lagarrigue, 1969; Mason, 1970). It should be noted that the values for

the ions in Orchestia haemolymph are similar to that of various Ligia species (Parry, 1953; Lagarrigue, 1969). Ligia inhabits a level on the shore comparable to that of Orchestia (see chapter 2). It may be that the immediate surroundings of these animals may be "salt-laden" due to evaporation. If this were so, it would help to explain the variance in blood sodium concentration measured in chapter 6 and the present chapter.

Robertson (1949) found that more active crustacean species had low blood magnesium concentrations. He subsequently put forward the theory that decreased blood magnesium and increased activity were causally related (Robertson, 1953). In this paper he quotes examples of studies in which neuromuscular transmission had been impaired by increasing haemolymph magnesium. Both Orchestia species have blood magnesium concentrations between that of Robertson's "active" (Nephrops, 17.8 meq.l^{-1} Mg^{2+}) and "passive" (Lithodes 104.4 meq.l^{-1} Mg^{2+}) Crustacea. A recent study on the intertidal prawn, Palaemon elegans seems to strengthen the evidence for a relationship between low magnesium concentrations and increased activity rates (Ramirez de Isla Hernandez and Taylor, 1985).

Robertson (1953) also found that the effect of the magnesium ion was counteracted by the presence of calcium in the blood. In spider crabs, the Ca^{2+}/Mg^{2+} ratio varied between 0.19 and 0.31 while in the remaining decapods the Ca^{2+}/Mg^{2+} ratio was 0.39-2.0. The Ca^{2+}/Mg^{2+} ratios calculated for O. gammarellus and O. mediterranea were 0.68 and 0.59 respectively, placing both talitrids in the latter category. In the more terrestrial arthropods such as woodlice, scorpions and spiders (and some fresh water crayfish), the blood calcium is at least 2.5 times greater than the blood magnesium (Gondko et al., 1984). This is far greater than was found in either of the talitrids examined.

It is expected that a halophilous species would display, for the main ions, a concentration higher than a non-halophilic species (Laggarique, 1969). This is borne out in a comparison of O. gammarellus from the extreme edge of its zone with various fresh-water amphipods and more fully terrestrial isopods such as Porcellio species (Lagarrique, 1969). In Oniscus assellus and Porcellio scaber, all of the ions measured, together with the osmotic pressure of the blood, were approximately half that of the semi-terrestrial Ligia species (Lagarrique, 1969). It could be suggested, therefore, that the observed change in O. gammarellus haemolymph, taken from animals at the extremes of their vertical distribution, was the result of a simple dilution effect.

5.4.2 Blood pH

The in vivo pH values of haemolymph from both Orchestia spp. fall well within the range of values reported for other crustaceans (Mangum and Shick, 1972; Sevilla and Lagarrique, 1979; Jokumsen et al., 1981). This is even allowing for variations in experimental temperature; differences of which will inevitably affect pH (Howell et al., 1973). The results reported here strengthen the suggestion that there is a degree of homogeneity in the blood pH of the Crustacea (Sevilla and Lagarrique, 1979).

5.4.3 Oxygen carrying capacity

The total oxygen carrying capacity (dissolved + bound oxygen) of the blood of both species was quite low (1.23 $ml.100ml^{-1}$) and did not differ significantly between O. gammarellus and O. mediterranea. This value compares closely with the value of 1.8 $ml.100ml^{-1}$ in the aquatic isopod Mesiodothea entomon (Hagerman and Oksama, 1985) and 1.61 $ml.100ml^{-1}$ in the supralittoral species, Ligia oceanica (J.I.S. ,

unpubl. obs.), the only species of isopod for which we have information. Much more is known, however, about the oxygen carrying capacity of the blood of decapod Crustacea (see review of Mangum, 1983a). Within the decapods, the oxygen carrying capacity of the blood is characteristically low, especially amongst the aquatic species (range 1 - 2 $ml.100ml^{-1}$), although slightly higher values have been recorded for some natant species (Hagerman and Weber, 1981; Weber and Hagerman, 1982; Morris et al., 1985). It has been suggested that the low oxygen carrying capacities of the blood of aquatic decapods may result from problems of fluid balance which require that the haemocyanin concentration of the blood is kept at low levels (Mangum and Johansen, 1975; Mangum, 1980). The situation in terrestrial crabs appears to be rather different since, in the majority of species studied so far, the oxygen carrying capacity of the blood is higher than in aquatic species e.g. 2.9 $ml.100ml^{-1}$ in Cardisoma carniflex (Burggren and McMahon, 1981) and 3.5 $ml.100ml^{-1}$ in Coenobita clypeatus (McMahon and Burggren, 1979). It is interesting, therefore, that the oxygen carrying capacity of the blood of Orchestia gammarellus, a semi-terrestrial species, is low and is closer to that of the aquatic decapods. Further information on other amphipod genera, from a range of environments is needed, however, before the full ecological implications of these differences can be appreciated.

5.4.4 Lack of haemocyanin in O. mediterranea

It is known that in many decapods the haemocyanin concentration of the blood can fall to extremely low levels in recently moulted animals (Zuckerkandl, 1957; Lewis and Haefner, 1976). This may explain the lack of haemocyanin in the blood of O. mediterranea that was occasionally observed during this study. Many talitrids are known to undergo a synchronised moult (Charniaux-Cotton, 1957) and it was noted

that the exoskeletons of the majority of animals sampled on this occasion were soft, indicating that moulting had recently taken place. It follows that during the time that haemocyanin is absent from the blood, the animal's respiratory demands are met by oxygen transported in solution by the blood. The reduced ability of the blood to transport oxygen may be partially compensated by the increased permeability of the body surface following moulting and by the fact that Orchestia, like many other crustaceans, remains quiescent after moulting (Charniaux-Cotton, 1957).

5.4.5 Oxygen affinity and the Bohr effect

The other respiratory properties of the blood of O.gammarellus and O. mediterranea, such as oxygen affinity, Bohr factor and cooperativity fall within the ranges reported for the majority of decapods (see review of Mangum, 1983a). Comparisons with other amphipods are again impossible due to the complete lack of data for this group. The only other crustacean group apart from the decapods for which information is available is the Isopoda but even here few data exist. Such comparisons should be treated with caution, however, since recent work has indicated that lactate can affect haemocyanin oxygen affinity, at least in decapods (Truchot, 1980; Graham et al., 1983; Mangum, 1983b; Bridges et al., 1984), and in the majority of previous studies information on the lactate concentration of the blood is lacking. Without such information it is difficult to make meaningful interspecific comparisons.

It is also essential that the oxygen tensions of the pre- and post-branchial blood are known if the role of haemocyanin in blood oxygen transport in these animals is to be fully understood. Unfortunately, due to the small size of these amphipods it was impossible to obtain this information. Nevertheless, even without

these data, comparisons of the respiratory properties of the blood with those of other animals can still be instructive.

The haemocyanin of both O. gammarellus and O. mediterranea exhibits a moderately high oxygen affinity compared with the values for P_{50} recorded in the decapods (Mangum, 1983a). There is evidence in the literature that the possession of a respiratory pigment having a high oxygen affinity is often correlated with an animal's ability to survive at low ambient oxygen tensions. Orchestia mediterranea occurs under stones in the eulittoral zone and it is known that conditions within the pools of water which remains under such stones at low tide are often hypoxic (Agnew and Taylor, 1986). This is also the habitat of the high shore gammarid Echinogammarus pirloti, which under hypoxic conditions, exhibited a high degree of respiratory independence with a critical Po_2 value as low as 15 Torr (Agnew and Taylor, 1985). The absence of such respiratory independance in O. mediterranea (Walshe-Maetz, 1952, 1956), coupled with the animals preference for drier habitats during low tide (J.I.S. unpubl. obs.) would suggest that prolonged hypoxic exposure does not play a major role in the biology of these animals. Nevertheless, the moderately high affinity of O. mediterranea may be advantageous, especially during the summer when, as a result of large amounts of decaying algae on the shore, the Po_2 of the incoming tide can be very low (Agnew and Taylor, 1986). In contrast, although O. gammarellus is known to be quite tolerant of hypoxia (Moore and Francis, 1986a), it appears that it is rarely exposed to hypoxia even in the dense algal mats in which it is frequently found (Moore and Francis, 1985a).

The oxygen affinity of the blood of Orchestia spp. is much higher than that of the terrestrial isopods, Porcellio laevas and Armadillium spp. (P_{50} = 31 - 68 Torr at pH = 7.65, 20^0C) (Sevilla and Lagarrique, 1979). It is interesting, however, that the blood of Ligia italica,

which occupies a position on the shore corresponding to that of O. gammarellus, has an oxygen affinity (P_{50} = 16 Torr at pH 7.65, 20^{0}C) comparable to that of the semi-terrestrial amphipods examined here (Sevilla and Lagarrique, 1979), although Terwilliger et al. (1979) found a slightly lower oxygen affinity in the closely related L. exotica.

Both Orchestia spp. have moderately high Bohr values of around 0.8 which are comparable with many aquatic decapods (Mangum, 1983a). From these values we can infer that at the level of the tissues in these amphipods, approximately 0.8 M of protons are bound per mole of oxygen released by the pigment. In this instance the Bohr effect favours oxygen unloading at the tissue level even at small pH differences. The low oxygen carrying capacity of Orchestia haemocyanin mentioned above, means that approximately half of the oxygen delivered to the tissues is transported in physical solution. Therefore protons bound to the pigment at the tissues during oxygen unloading are reduced compared to proton production. This in turn will favour lower pH values at the level of the tissues as compared with haemolymph pH at sites of oxygen uptake. In vivo pH changes, associated with an increase in activity may also augment unloading of oxygen at metabolising tissues.

Young (1972) postulated that among crabs, increasing 'terrestrialness' was correlated with reductions in haemocyanin oxygen affinity and in the magnitude of the Bohr effect. Since Young's study, however, more data have become available for decapods from a variety of habitats and some doubt has been expressed about this hypothesis (Mangum, 1982). Nevertheless, the work of Sevilla and Lagarrique (1979) on an ecological series of isopods has indicated that such a correlation may well exist among that group. This subject will be

considered more fully in chapter 7.

5.4.6 Pigment temperature insensitivity

Many poikilothermic animals inhabiting environments that are subject to considerable temperature variation possess respiratory pigments that show an adaptive decrease in temperature sensitivity (Young, 1972; Rutledge, 1981; Jokumsen and Weber, 1982; Arp and Childress, 1985; Morris et al., 1985; Morris and Bridges, 1985). Temperature insensitivity of the respiratory pigment can be regarded as adaptive in these animals since the reduction in oxygen affinity of the pigment with increasing temperature, which is also compounded by the associated effect of temperature on blood pH (Howell et al., 1973), could seriously affect oxygen loading at the respiratory surfaces and reduce the oxygen reserve in the venous blood. It is perhaps not surprising, therefore, that the haemocyanin of both O. gammarellus and O. mediterranea has a low temperature sensitivity since both these species may be subject to considerable temperature variation, in spite of the fact that they may be "buffered" from the more extreme diurnal changes in temperature under blankets of decaying algae (Backlund, 1945; Moore and Francis, 1985a).

5.4.7 Further studies on O. gammarellus

The important difference in the oxygen affinity of the haemocyanin from O. gammarellus collected from different locations of the same shore is particularly noteworthy. The reduction of the oxygen affinity of AHW animals measured in vitro can be attributed primarily to the changes in ionic composition of the blood since it is now well established that the ionic environment of the blood can have important effects on its oxygen transporting properties (Spoek, 1967; Truchot, 1975; Brouwer et al., 1978; Miller and Van Holde, 1981; Mason et al., 1983).

The blood ionic composition of animals from high on the shore (AHW animals) was very different from that of animals in the middle of their zone (BHW animals). The reduction in the concentration of nearly all the major ions of the blood of AHW animals probably resulted from being exposed to fresh water run-off at the top of the shore and not being covered by the sea, even at spring tides. It is more difficult to explain the reduction in the pH of the blood of animals from slightly higher on the shore (AHW animals). Since there was no significant difference in the protein concentration of the blood of AHW and BHW animals, it is unlikely that the buffering capacity of the blood varied between them (but see next chapter). It is possible however, that the AHW animals, which were probably subjected to greater 'stress' in the form of ionic regulation, had a higher rate of oxygen consumption. This was not presently examined but is a common response of many marine animals on exposure to low salinity. If this were the case, the resulting increase in CO_2 production could account for the reduction in blood pH especially since elimination of CO_2 is more difficult in terrestrial and semi-terrestrial animals than in aquatic animals (O'Mahoney and Full, 1984). Changes in the microenvironment of the blood associated with hyposaline exposure are considered more fully in the next chapter.

5.4.8 Effect of Calcium on oxygen affinity

The dialysis experiments demonstrated that the reduction in the oxygen affinity of the blood of AHW animals was probably due to low Ca^{2+} ion concentration in the blood. A number of studies have shown that divalent ions generally increase oxygen affinity (Larimer and Riggs, 1963; Truchot, 1975; Brower et al., 1978; Miller and Van Holde, 1981; Mason et al., 1983; Terwilliger et al., 1979) either because

they stabilize the high affinity configuration of the respiratory pigment or because they promote polymerisation to a molecular form that has a higher oxygen affinity than its component subunits (Mangum, 1983a). The value for the factor describing calcium sensitivity, $\Delta \log P_{50}/\Delta \log [Ca^{2+}]$ (Larimer and Riggs, 1963) is moderately high in O. gammarellus at -0.41. This is closer to the value of -0.28 calculated for Carcinus maenas (Truchot, 1975) than the figure of -0.82 given for the blue crab, Callinectes sapidus by Mason et al. (1983). It also appears that Ca^{2+} ions have a greater effect on oxygen affinity than Mg^{2+} ions (Miller and Van Holde, 1974), but Mg^{2+} ions also affect the size of the Bohr effect (Truchot, 1975). Unfortunately, there was insufficient blood available during this present study to investigate the effect of Mg^{2+} ions on the respiratory properties of the haemocyanin of O. gammarellus. The results of the dialysis experiments appeared to show, however, that the reduction in oxygen affinity of the blood of AHW animals was due almost entirely to the low concentration of Ca^{2+} ion in their blood. In those experiments in which the blood of AHW animals was dialysed against a physiological saline having an ionic composition identical to that of the blood of AHW animals but in which the Ca^{2+} concentration had been increased to the same level as in BHW animals, the oxygen affinity of the blood was restored almost completely to that of BHW animals.

The results of these experiments indicate that Ca^{2+} ions may have a similar effect on blood oxygen affinity in both amphipods and decapods. The situation in the isopods, however, appears rather more confused. Sevilla and Lagarrique (1979), in a comparative study of the haemocyanin from several species of terrestrial and semi-terrestrial isopods, found that in Ligia italica, Tylos latreillei and Armadillum officinalis divalent ions increased oxygen affinity but in Porcellio laevis, Armadillidium granulatum and A. vulgare they resulted in a

reduction in oxygen affinity. Also Van Holde and Brenowitz (1981) found that divalent cations had no effect at all on the oxygen binding properties of the haemocyanin of the giant isopod, Bathynomus giganticus.

5.4.9 Conclusions

The present study is one of the first to deal with oxygen transport in amphipods. An almost total lack of information on the respiratory properties of amphipod haemocyanin precludes detailed discussion of the ecological significance of the haemocyanin properties in O. gammarellus and O. mediterranea in relation to other amphipod families. One of the most interesting points to have emerged from this study is the fact that the oxygen transporting properties of the blood of these two talitrid species are so similar despite their ecological differences. This raises a number or questions concerning the respiratory adaptations of amphipods to semi- and fully terrestrial life which will be considered in the last chapter.

The oxygen transporting properties of the blood of Orchestia species show a number of similarities with those of the blood of isopod and decapod Crustacea ie. it is conservative within the Crustacea (Mangum, 1983a). More information on other taxa is necesary to test this hypothesis and to increase our understanding of the physiological and ecological significance of interspecific differences in the respiratory properties of haemocyanins

5.5 Appendix : Notes on the appearance of blood from Orchestia spp. and other amphipod species.

The blood of both O. gammarellus and O. mediterranea is normally a dark blue colour. Under the microscope, the blood can be seen to contain small corpuscle-like fragments and numerous lipid globules,

the latter containing a purple pigment. The plasma itself appears colourless. The dark blue pigmentation is due not to haemocyanin but to a chromoprotein of the carotenoid type (Rawlinson, 1937; Goodwin, 1960; Wieser, 1965). It shows no appreciable change in colour when it is deoxygenated. As suggested by Rawlinson (1937) the pigment turns orange red when heated. The addition of sodium dithionate had a similar effect.

Measurements of the absorption spectra were made to clarify the presence of haemocyanin in the blood and to characterise its absorption spectra. Absorption spectra over the range 250 - 650 nm were determined for the blood of O. gammarellus using a Pye-Unicam S.P. 8000 scanning spectrophotometer. 10 ul of blood was diluted with 890 ul of a physiological saline, the ionic composition of which was based on the previously determined ionic concentrations of the blood. The diluted samples were analysed at room temperature in 1 cm pathlength (quartz, 1.00 ml capacity) spectrophotometer cells. Absorption spectra for the blood show the occurrence of a typical protein absorption peak at around 270 -295 nm and a smaller peak between 330 and 340 nm. The smallest peak (the copper peak) disappeared on deoxygenation of the diluted blood. This confirmed that haemocyanin was present in O. gammarellus haemolymph. No absorption bands were found above 340 nm. This may have been due, however, to the magnitude of the dilution factor employed being so great as to obscure other bands.

The whole blood absorption spectrum for Orchestia was similar to that found for the Decapoda (Miller and Van Holde, 1974), the oniscoidean Isopoda (Sevilla, 1978), Gammarus locusta and G. pulex representing the Amphipoda (J.I.S. unpub. obs.) and other molluscan and arthropodan haemocyanin solutions as measured by Nickerson and Van

Holde (1971). The extinction coefficient per milligram protein ($E_{1\ cm.mg^{-1}}$: Value for blood protein taken as 22 $mg.ml^{-1}$) for the protein peak at 280 nm is very similar at 14.2, to the value of 14.8 calculated for the haemolymph of Palaemon elegans (Taylor and Spicer, unpublished).

The determination of oxygen dissociation curves by spectrophotometric means was rendered impractical by the opaque nature of undiluted Orchestia haemolymph. This is due to the presence of chromoprotein (see earlier). After moult, however, the strong blue pigmentation disappears and the blood becomes very pale. The lipid globules, while still present, are almost colourless and give the haemolymph a faint orange tinge. The masking of haemocyanin in the blood by chromatoprotein as found in Orchestia gammarellus, O. mediterranea, O. cavimana and Arcitalitrus dorrieni, but is not such a permanent feature of the haemolymph of Talitrus saltator or Talorchestia deshayesii. In these animals, the haemolymph is often clear though with a distinct bluish tinge. The tint disappears upon deoxygenation. Alikhan et al. (1974) has reported that the haemolymph of the fresh-water Gammarus fossarum is bluish-yellow, turning blue when exposed to air. This is similar to the blood of the sandhoppers mentioned above and also that of Gammarus locusta. Gammarus pulex on the other hand, has a very dull blue-grey haemolymph which is not altered in its coloration by deoxygenation.

On puncturing the exoskeleton of Orchestia (also Talitrus and Talorchestia), the blood flows quickly from the incision which suggests that blood pressure may be quite high. This is most marked in post-moult individuals. This feature is not so noticeable in Gammarus pulex, G. locusta or even the fully terrestrial talitrid Arcitalitrus dorrieni.

CHAPTER 6

EFFECT OF HYPER- AND HYPO-SALINE EXPOSURE ON THE BLOOD AND TISSUES OF *O. GAMMARELLUS* IN THE FIELD AND IN THE LABORATORY

6.1 INTRODUCTION

Osmoregulatory mechanisms have been studied in many invertebrate groups and the results reviewed extensively (Schoffeniels, 1976; Gilles, 1979; Gilles and Pequeux, 1983). In the Crustacea, hyposaline exposure results in a decrease in blood ions and also a decrease in the osmotic concentration due to loss of blood ions together with an inward movement of water. Intracellular osmolarity during hypo-osmotic stress is regulated by a decrease in intracellular free amino acids (FAA) together with other low molecular weight nitrogenous solutes.

To date, there have been few experimental studies concerned with the effects of salinity on talitrid amphipods. This is in sharp contrast to the work that exists on a number of euryhaline aquatic amphipod species (McLusky, 1968; Preece, 1970; Dorgelo, 1977; Bulnheim, 1979). The information that exists for the Talitridae is derived almost exclusively from semi-terrestrial species (Wildish, 1970a,b; Bock, 1967; Backlund, 1945; Marsden, 1980; Moore and Francis, 1985a, 1986a)with the exception of two values for blood osmotic pressure: one from an unidentified (Dr. C. Little, pers. comm.) euterrestrial landhopper species (Little, 1983) and the other from a supralittoral Talorchestia sp. (Moloney and Nicolson, 1984).

There have been a few experimental studies dealing with the gross effects of salinity on various talitrids. Wildish (1970b), examining the distribution of Orchestia sp. in the Medway estuary, found that the median lethal concentration of dilute seawater (0.4 - 1.8 ‰ Cl') for O. gammarellus was near the limiting isohaline at high water (1 - 2 ‰ Cl') in the estuary. The tolerance limit of O. mediterranea was reached at 5.8 - 6.4 ‰ Cl' at high water. Two distinct morphs of the latter species exist (Wildish, 1970c), and the more marine of the two

(morph mediterranea as opposed to morph aestuarensis) shows sublethal effects eg. increased intermoult period and egg mortality, due to the low chlorinity of the substrate and of the food (<8.1 ‰ Cl^{-}). It appears that morph aestuarensis, is in some way better adapted to lower salinity than morph mediterranea. In general, most European and American sea-shore talitrids are rather indifferent towards salinity (with some notable exceptions, see Bousfield, 1958; Bowers, 1964) but die if they are immersed in fresh water (Smallwood, 1903, 1905; Backlund, 1945; Dahl, 1946; Den Hartog, 1962; Bock, 1967; Hurley, 1968).

Survival time at reduced salinities is decreased by an increase in temperature (Bowers, 1964), although there are differences in the survival times of summer and winter populations of Transorchestia (=Orchestia) chiliensis (Marsden, 1980). In his study of the wrack fauna of Sweden and Finland, Backlund (1945) claimed that both O. gammarellus and Platorchestia platensis have low salinity preferences (around 3 ‰) when exposed to a salinity range of 0 to 200 ‰ ; but when he placed them within the confines of a more limited salinity range (0 - 10‰) neither of the species exhibited any marked salinity preference. Embryos of O. gammarellus can also withstand changes in external salinity. At lower salinities, although the rate of development remains constant, the number of hatched juveniles declines with chlorinity (Vlasblom and Bolier, 1971).

Bousfield (1958) states that one of the principal modifications accompanying the aquatic/terrestrial transition in the amphipods is the increased efficiency of osmoregulation. Unfortunately he presents no data in the paper to substantiate his claim. Some recent work on O. gammarellus however, has shown that this animal possesses fairly good powers of osmoregulation (Moore and Francis, 1985a). The ability of O. gammarellus to hyper-regulate over a wide range of dilute salinities

(see also Bock, 1967 on P. platensis) can be related to its considerable penetration into estuaries as mentioned above. It has also been suggested that the dependence of littoral talitrids on external chloride ions may be an important factor in the restriction of these animals to maritime regions (Bousfield, 1958; see also Friend and Richardson, 1986). It has been shown experimentally, however, that it may not be the chloride ion but the associated sodium ion that is more likely to be the limiting factor (Moore and Francis, 1986a). It has been suggested (Friend and Richardson, 1986) that landhoppers (Group IV;1, Bousfield, 1984) have developed greater powers of osmotic and ionic regulation than have terrestrial beachflea genera (Group II, Bousfield, 1984) enabling the former to penetrate further inland. This has yet to be subjected to experimental verification.

This study sets out to examine the effects of long term hypo- and hyper-saline exposure on O. gammarellus at the level of the blood and tissues. Employing both laboratory and field data, changes in the pH, blood sodium and protein concentrations and the total free amino acid pool of the species were assessed. The results are of interest for two reasons: a) to discover the homeostatic mechanisms involved when O. gammarellus is exposed to changes in environmental salinity and compare the results with those for other crustacean groups and b) because they provide a background to the description of the blood gas combination properties of the haemolymph in the previous chapter.

6.2 MATERIALS AND METHODS

6.2.1 Laboratory studies

Orchestia gammarellus was collected by hand from beneath wrack beds at H.W.M. on Ballochmartin Bay, great Cumbrae Island. The animals were transported back to the laboratory alive in plastic bags containing some of their native substratum and some fresh seaweed. On arrival they were transferred (30 animals per dish) to a number of crystallising dishes (100 ml capacity). The dishes were covered with muslin to prevent the animals escaping, and kept moist by the addition of filter-paper soaked in seawater of an appropriate ionic composition. This filter-paper was separated from the animals by a sheet of plastic gauze. Each dish was supplied with excess food in the form of agar (Zobell's 2216E) made up with artificial sea salt (Tropic marine) solutions of a range of different salinities (0, 5, 10, 20, 30 and 40 ‰). Orchestia gammarellus were kept under these conditions for 72 hours in a controlled temperature room held at 10^0C.

At the end of the experimental period, animals were removed and haemolymph samples were taken using the method outlined in Chapter 5. The haemolymph was immediately transferred to a 1.5 ml Eppendorf tube stored on ice before being deep frozen at -20^0C. The bled body of the animal was then quickly immersed in liquid nitrogen for 10-20 seconds and stored in a labelled vial at -20^0C. Only intermoult males as determined by the criteria of Charniaux-cotton (1957) were used; animals that had moulted during the experiment were discarded.

Total blood protein and sodium ion concentration were also determined using the methods described in chapter 5. The total FAA was determined for individual animals as follows. The whole animal was freeze-dried at -70^0C and then homogenised in 0.5 ml of perchloric

acid at 5^0C. Following centrifugation (10,000 g), the resultant supernatant was neutralised using $KHCO_3$. Total FAA was then assayed using a ninhydrin method (Moore and Stein, 1948) and expressed as µmol total FAA. g^{-1} dry body weight. Alanine was run as a standard.

6.2.2 Field studies

Large male O. gammarellus were collected from a number of different sites at the one location at Ballochmartin Bay (see chapter 2 for details). The sites were as follows, (a) well above the H.W.M., beneath stones and debris lying on grass and dry ground, (b) just at H.W.M., beneath a dense wrack bed, lying on a cobble beach, (c) beneath stones and rocks at the level of Pelvetia canaliculata four hours after the high tide, and (d) similar to the location (c.) except that P. canaliculata had been replaced by Enteromorpha sp. which was taken to be indicative of fresh-water influence. The collections were made on three consecutive days in summer (average air temperature = 21^0C), the weather being fine but overcast.

On removal of animals to the laboratory, blood was extracted immediately (within 2 hours of capture) using the method outlined in chapter 5. Only animals which yielded 10 µl+ of blood were considered. 10 µl of haemolymph was diluted with 990 µl of double distilled water. Blood protein was assayed using 50 µl of this dilution by the Coomassie blue method outlined in chapter 5. The remaining volume was used to determine the blood sodium concentration, using an E.E.L. flame photometer and an appropriate range of sodium standards.

6.3 RESULTS

6.3.1 Laboratory studies

As expected, male intermoult O. gammarellus were found to be euryhaline (Table 6.1). After 72 h there was very little mortality at all test salinities with the exception of 0 and 5 ‰. There was marked swelling of the gills in animals which had died from exposure to the lower salinity media.

The sodium ion concentration in the haemolymph of O. gammarellus was very well regulated over the range 5 - 40‰ at 10^0C (Fig. 6.1). The calculated isoionic concentration was 350 $mM.l^{-1}$. The animal hyper-regulated at reduced salinities but hypo-regulated when exposed to environmental salinities > 30 ‰.

Total blood protein was seen to vary inversely with acclimation salinity (Fig. 6.2). There was an increase of 35% recorded after 3 days on transfer of 30 ‰ acclimated animals to 10 ‰. Conversely total FAA varied directly with acclimation salinity (Fig. 6.3) falling from 101.9 $\mu mol.g^{-1}$ at 30 ‰ to 64.7 $\mu mol.g^{-1}$ at 10 ‰. There was no significant difference in total FAA between 30 ‰ and 40 ‰ or 5 ‰ and 10‰ .

The pH of in vivo haemolymph samples, at 10^0C were seen to vary directly with acclimation salinity after 3 days exposure (Fig. 6.4). There was a drop of 0.15 pH units between 30 ‰ and 10 ‰ acclimated animals, but this reduction was more pronounced at 0 ‰ where the haemolymph pH was 7.401, ie. approximately 0.5 pH units below the blood pH of 30 ‰ acclimated animals.

TAB. 6.1 Mortality after 72 h of O. gammarellus presented with food of different salinities (n = 90 at each salinity).

Salinity (‰)	Percentage mortality
40	3
30	5
20	1
10	5
5	10
0	23

FIG. 6.1 The concentration of Na^+ ions in the haemolymph of *O. gammarellus* presented with food of different salinities at 10^0C. Analyses were carried out on pooled blood samples (100 µl) with n = 3 for each salinity.

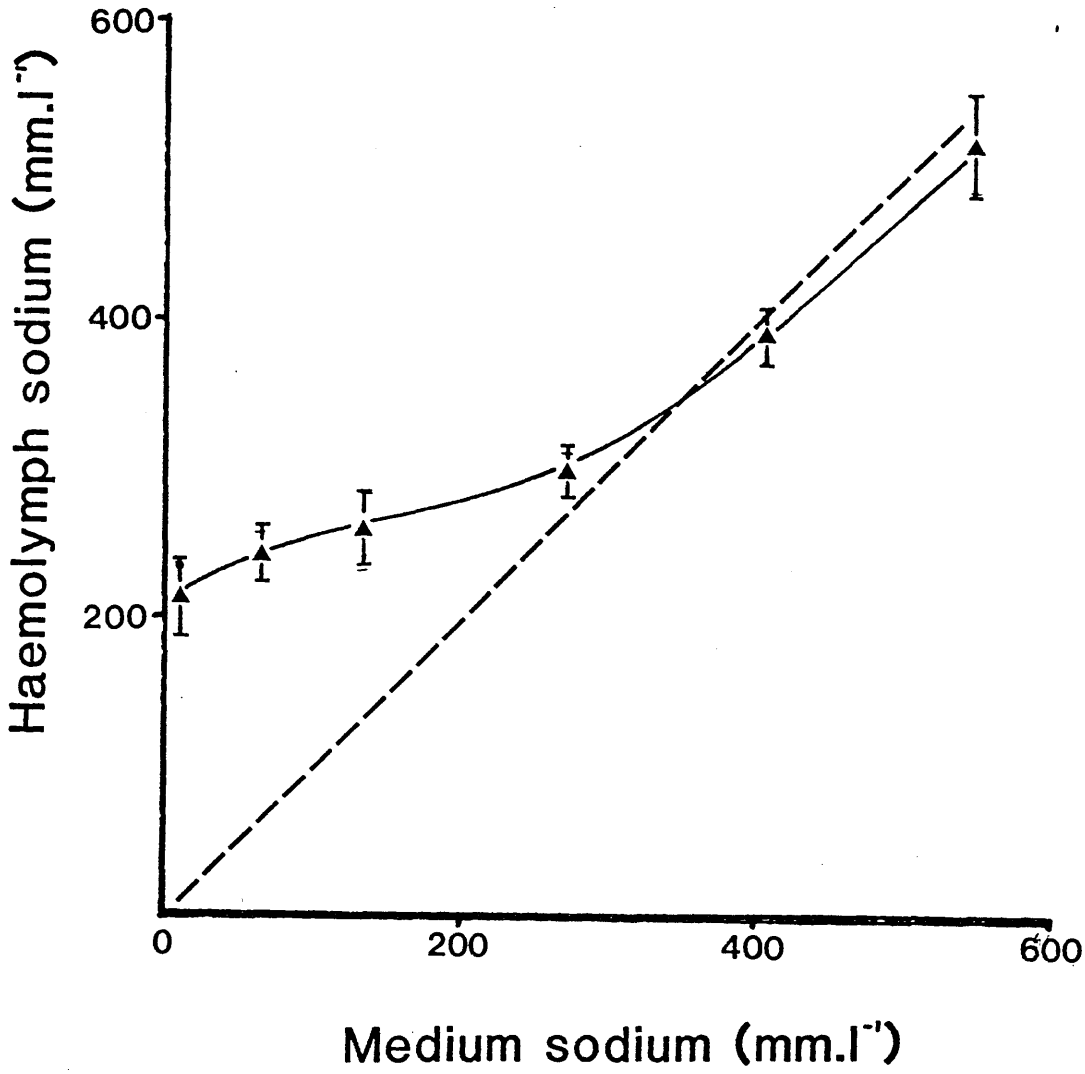

600
400
200
0
200
400
600
Haemolymph sodium (mm.l⁻¹)
Medium sodium (mm.l⁻¹)

FIG. 6.2 The total blood protein and oxygen carrying capacity of haemolymph from O. gammarellus presented with food of different salinities at 10^0C. The oxygen carrying capacity determinations were carried out on pooled blood samples (n = 2) for each salinity.

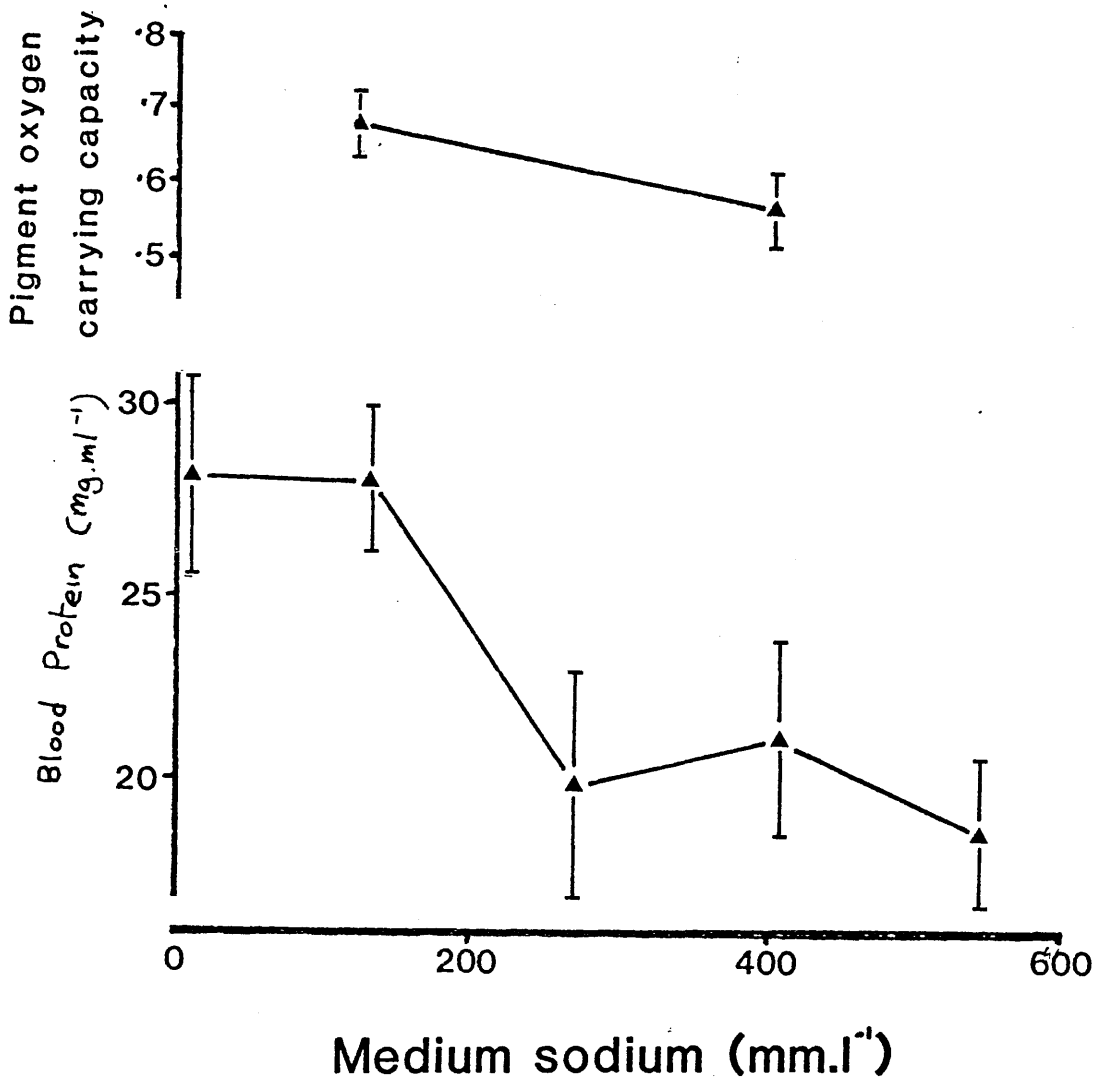

Pigment oxygen carrying capacity
·8
·7
·6
·5
Blood Protein (mg.ml⁻¹)
30
25
20
0
200
400
600
Medium sodium (mm.l⁻¹)

FIG. 6.3 Total free amino acid (FAA) content of individual O. gammarellus exposed to food of different salinities at 10^{0}C. (n = 10 for each salinity tested).

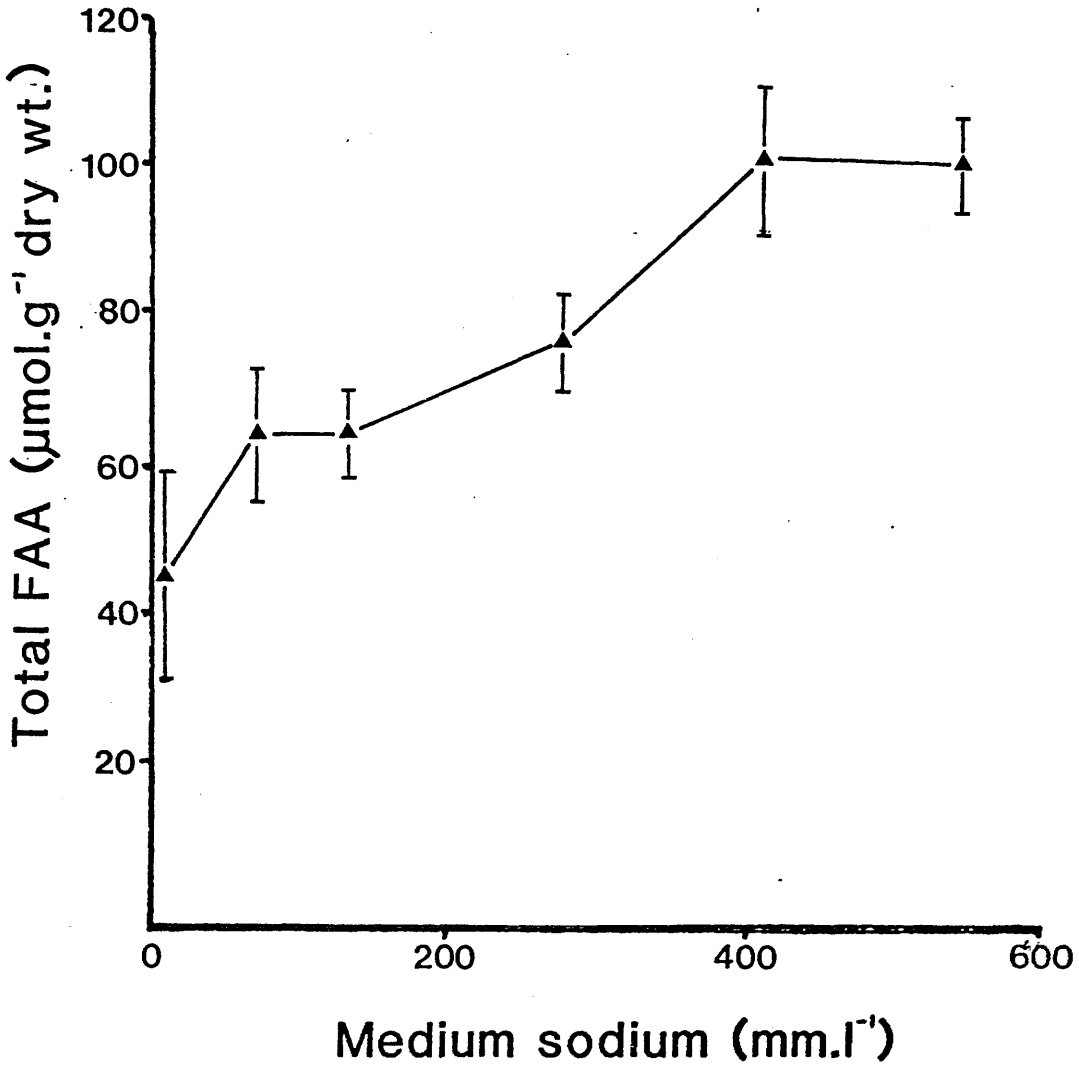
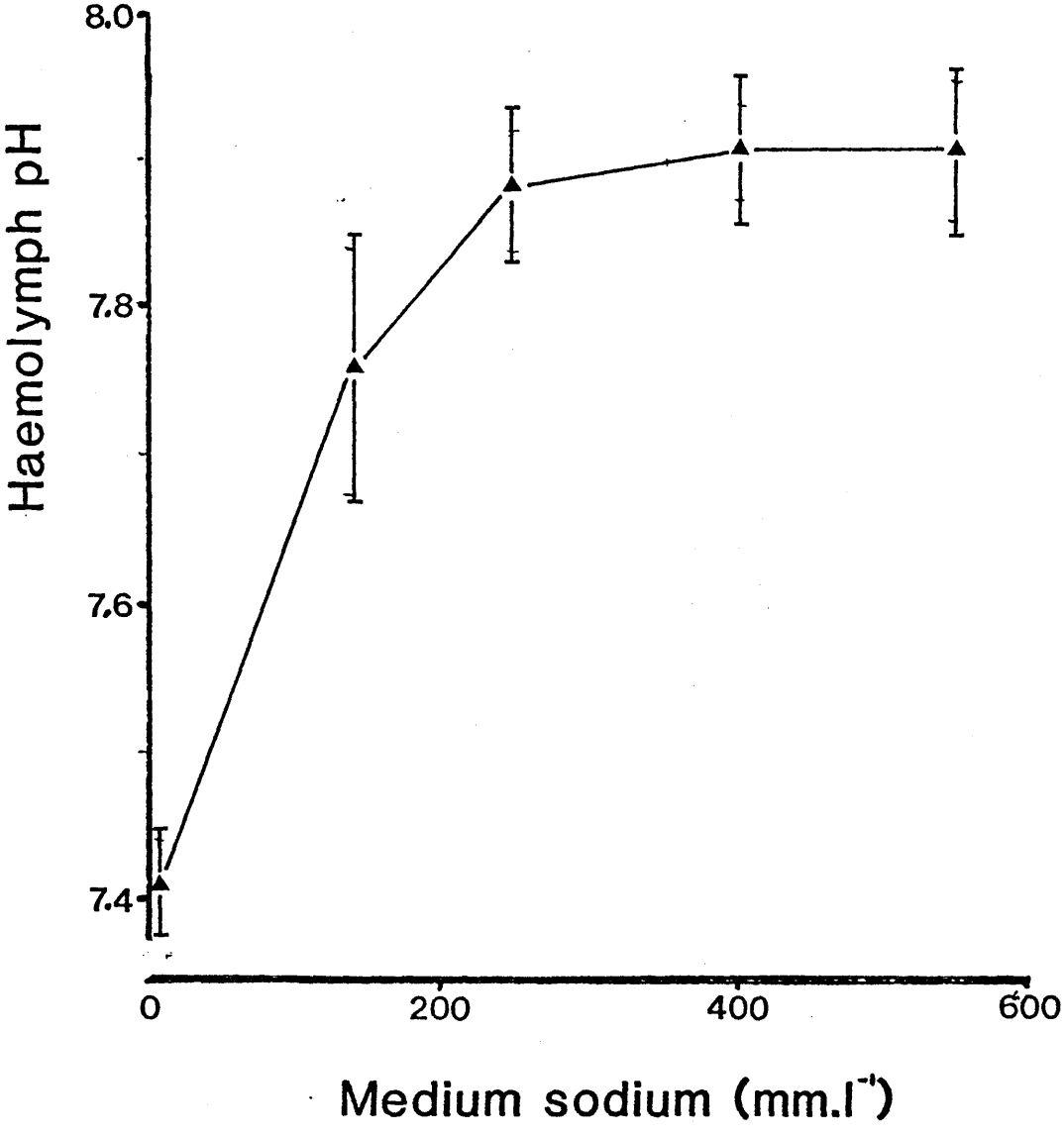

Total FAA (µmol.g⁻¹ dry wt.)
120
100
80
60
40
20
0
200
400
600
Medium sodium (mm.l⁻¹)

FIG. 6.4 The *in vivo* pH of the haemolymph of *O. gammarellus* exposed to food of different salinities at 10^{0}C. Determinations (n = 5 at each salinity) were carried out on individual animals.


8.0
7.8
7.6
7.4
Haemolymph pH
0
200
400
600
Medium sodium (mm.l⁻¹)

6.3.2 Field studies

The relationship between blood protein and sodium ion concentrations of the haemolymph from animals collected from (a) above the high water mark and (b) at the high water mark beneath wrack is shown graphically in Figure 6.5. The protein concentration of the haemolymph of the former (which is equivalent to the AHW animals of chapter 5) was quite variable with values ranging from 24 - 33 mg protein. ml^{-1} blood. The sodium ion concentration was typically low < 300 $mM.l^{-1}$. Conversely, animals collected from beneath the wrack at high water showed similar blood/tissue traits, indicating a homogeneity of blood protein/Na ; the sodium ion concentration was significantly greater than in animals collected from above the high water mark and while the blood protein content was lower it was, in general, less variable.

Superimposed on Figure 6.5 is the regression line calculated for the same relationship between blood protein/Na^+ under controlled conditions in the laboratory. It is of interest that both sets of points on the graph (with the exception of some of the AHW points) clumped at either end of this regression line.

In Figure 6.6, the regression line relating blood protein to blood Na^+ content in laboratory acclimated animals has again been superimposed upon a similar plot of paired values for blood protein and Na^+ obtained from animals collected at identical heights on the shore at the bottom of the *O. gammarellus*' zone. In haemolymph taken from animals which were collected in the *P. canaliculata* zone, the scatter of points was similar to those representing samples from animals taken beneath the wrack bed (see above). They exhibited a high blood Na^+ but low blood protein concentration. Conversely, haemolymph

FIG. 6.5 The relationship between protein and Na^+ concentrations in the haemolymph of individual O. gammarellus, collected above (△) and below(▲) the high water mark, at the extremes of the species' vertical distribution on the shore (see text for details).

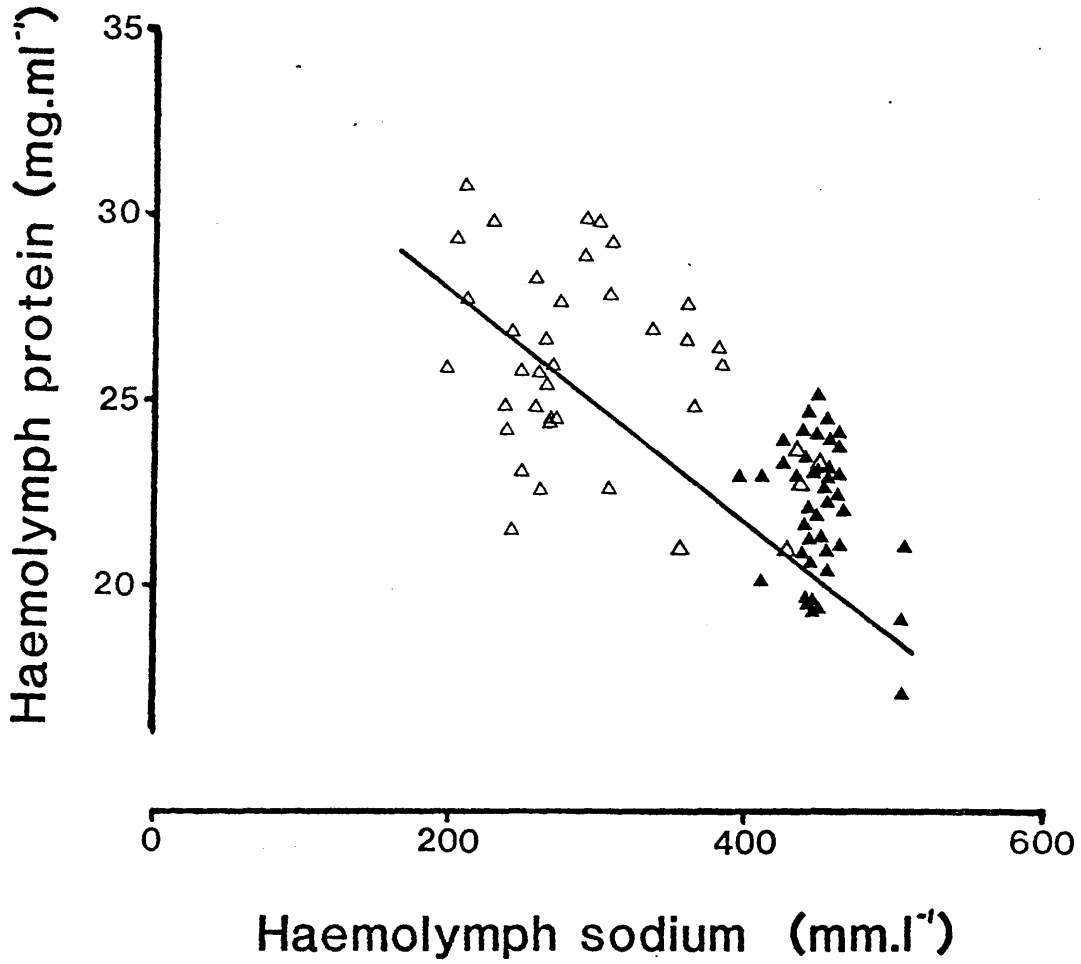
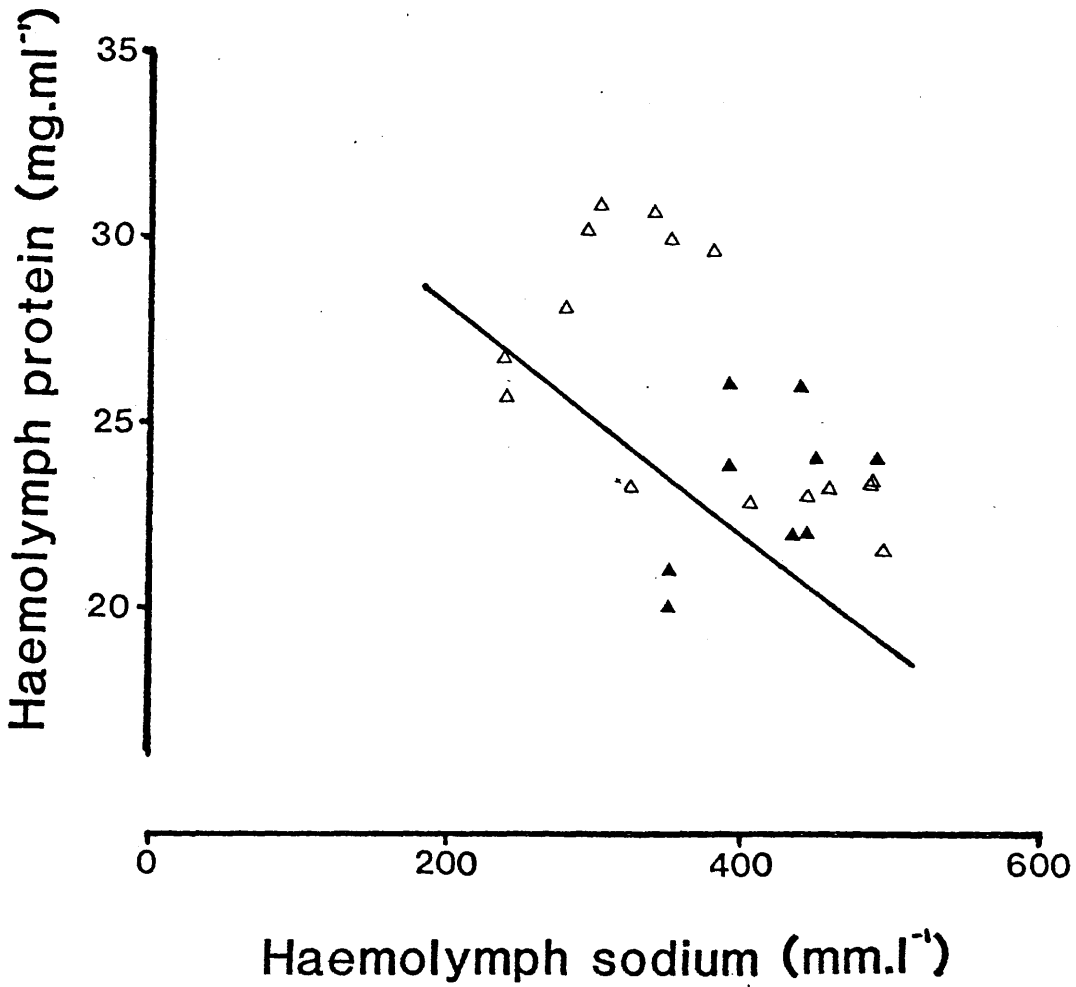

Haemolymph protein (mg.ml⁻¹)
35
30
25
20
0
200
400
600
Haemolymph sodium (mm.l⁻¹)

FIG. 6.6 The relationship between protein and Na^+ concentrations in the haemolymph of individual O. gammarellus collected beneath wrack, from just below the High Water Mark at locations c. (▲) and d. (△). (See text for details).


Haemolymph protein (mg.ml⁻¹)
35
30
25
20
0
200
400
600
Haemolymph sodium (mm.l⁻¹)

samples from animals exposed to greater fresh water run-off at the same level on the shore had, in general, low blood Na^+ and high blood protein. It is evident, however, that the microhabitats in this area must vary dramatically in their degree of fresh-water influence. This is shown in the large scatter of points encompassing, in many cases, the characteristic high Na^+/low protein combination of more 'stable' areas.

Preliminary experiments showed that it was possible to reverse the characteristic blood Na^+/protein combination found in haemolymph for both AHW and BHW animals. This showed clearly that the changes in these parameters were determined solely by the prevalent salinity microregimes encountered by O. gammarellus. The large variations encountered may reflect the diversity of microhabitats even in the same general area as regards salinity and other environmental factors (eg. temperature, relative humidity etc.).

6.4 DISCUSSION

6.4.1 Salinity tolerance

Large individuals of O. gammarellus were able to survive 72 hours exposure to a wide range of food salinities at 10^0C. This finding is in accord with the results of Moore and Francis (1986a) who investigated the salt tolerance of this species under conditions of immersion. They found that both small and large animals survived >96 hours in salinities between 1 - 60 ‰. Seemingly contrary to this present work in which most of the animals exposed to medium made up using fresh-water survived the duration of the experiment, Moore and Francis (1986a) found that when O. gammarellus were immersed in distilled water death occurred within 8 -24 hours. This discrepancy may be due to the different conditions of immersion and emersion in

each case, and may be indicative of a genuine difference in the animals' physiological response in each case. Many other euryhaline aquatic amphipods are able to tolerate such conditions of hypo- and hyper- saline exposure (Dorgelo, 1974; Agnew, 1985) and so this faculty, although it may be pre-adaptive, may not be directly related to the acquisition of semi-terrestrial habits in the Talitridae.

6.4.2 Haemolymph sodium regulation

It is known that wide variations in external sodium ion concentrations can be tolerated by O. gammarellus; indeed, this is considered to be the most important ion species to this animal (Moore and Francis,1986a). It was not unexpected then, that the pattern of regulation between 10 - 40 ‰ of this major blood cation was similar to that of the total osmotic concentration of the blood (Moore and Francis, 1985a). Furthermore, while the osmotic regulation begins to break down < 10 ‰ in this species (Moore and Francis, 1985a), the blood sodium concentration continues to be regulated to around 5 ‰ (or perhaps even lower). This feature further strengthens the view expressed above, that sodium is one of the most important ions to O. gammarellus (see chapter 7 for further discussion). Again, this highly developed ionic regulatory mechanism is not peculiar to the Amphipoda of the supra-littoral zone and is found in many other euryhaline amphipods (Shaw and Sutcliffe, 1961; McLusky, 1968; Sutcliffe, 1971 a,b) as well as other intertidal crustacean species (Zanders, 1980; Ramirez de Isla Hernandez and Taylor, 1985).

6.4.3 Mechanism of osmoregulation in O. gammarellus

No euryhaline species could tolerate changes in blood volume such as would result from hyper- and hypo-saline exposure, if their cells were simply to act as osmometers. Most euryhaline animals can maintain

relatively constant blood and cell volumes when adapted to a range of external salinities, by varying a number of the constituents of their haemolymph and body tissue.

In both pooled and individual haemolymph samples taken from O. gammarellus, the decrease in blood ionic concentration, as indicated by the fall in Na^+ ion concentration (see also chapter 5), is very often accompanied by an increase in the overall protein concentration of the blood. Such modifications following hypo-osmotic stress have been reported in other crustaceans (Siebers et al., 1972; Gilles, 1977; Pequeux et al., 1979; Taylor et al., 1986). As relative haemocyanin concentration of the blood is less variable than that found in typically marine amphipods (Wieser, 1965), this would suggest that with an increase in haemolymph protein there should be a corresponding increase in blood oxygen carrying capacity. Such an increase could be seen as adaptive, off-setting the effects of lower oxygen affinity in the haemolymph of AHW as compared with BHW animals. While this has been observed, its occurrence was not consistent. Although the possibility cannot be discounted, it is unlikely that changes in the blood protein concentration recorded in O. gammarellus associated with hypo-/hyper-saline exposure, are related solely to tissue hydration or changes in extracellular volume (Gilles, 1977). It may be that the increase in protein concentration is related to the reduction of the osmotic concentration of the blood as indicated by the fall in sodium ion concentration.

It is known that in the shore crab Carcinus maenas, haemocyanin synthesis is stimulated during hypo-osmotic stress (Boone and Schoffeniels, 1979). Also in the euryhaline crayfish Pacifastacus lenisculus, the free amino acid concentration

is increased at the expense of the respiratory pigment. In this case however, despite a reduction in oxygen carrying capacity, alterations of the oxygen binding characteristics of the haemolymph allow O_2 transport to carry on unimpaired (Wheatly and McMahon, 1981).

The decrease in total FAA during hypo-osmotic exposure in O. gammarellus has been observed in other intertidal crustacean species (Jeaniaux et al., 1961; Taylor and Spicer, in prep), and is also consistent with this theory. Free amino acids and other low molecular weight nitrogenous compounds have been implicated in cell volume regulation during hypo-/hyper- saline acclimation (see Introduction for references). During hypo-osmotic stress, such as may occur due to fresh-water run-off in the supra-littoral zone, membrane permeability could be altered and amino acids released into the haemolymph. These amino acids could then be used for blood protein synthesis (possibly in the hepatopancreas: see Ghiretti-Magaldi et al., 1973 on Carcinus maenas), the resultant blood protein acting as an amino acid store. It is also possible that the increased blood protein content would assist volume control by decreasing the osmotic swelling gradient which builds up when blood osmolarity is decreasing (Gilles, 1978).

An objection to this process occurring in Orchestia, however, is based upon the consequences of the deamination process. For as the free amino acid pool is reduced, blood ammonia increases; the ammonia molecule gains a proton and the blood pH should rise concurrently. In the case of Orchestia, the blood pH is decreased in animals exhibiting a reduced ionic composition. This conflict may be partially resolved by considering the time scale over which these changes in pH have been measured. In the rock pool shrimp Palaemon elegans, the blood pH increases within the first two hours of hyposaline exposure, but does not remain at this elevated level (Taylor and Spicer, in prep). The

blood pH change in Orchestia haemolymph was observed only after three days acclimation time. Also in the barnacle Pollicipes polymerus, where the internal pH decreases with acclimation salinity (Fyhn et al., 1972), the change resulting from additional CO_2 entering the haemolymph as a product of elevated aerobic metabolism in dilute media, may also be further compounded by problems associated with the elimination of CO_2 in an aerial environment (Little, 1983).

CHAPTER 7

THE PHYSIOLOGICAL ECOLOGY OF LAND INVASION BY THE TALITRIDAE

7.1 INTRODUCTION

7.1.1 Aim of chapter

Adaptation to life on land presents talitrid amphipods with a number of inter-related physiological problems. These problems are mainly concerned with water conservation, nitrogenous excretion, osmoregulation, respiration and locomotion. Although it is commonly held that little morphological or physiological change has been required by amphipods in response to the colonisation of land (Edney, 1960), different degrees of adaptation are certainly discernible between various amphipod families. Although he was not specific, Stebbing (1906a) recognised that there were"important distinctions" between Gammarus and Talitrus, but that these were 'bridged over' by the genus Hyale. King (1913) too, noted that, "in the orchestias....Hyale and Orchestia, physiological differentiation of genera as to endurance of exposure to the atmosphere made possible a land life for the latter." Even within the fully terrestrial landhopper genera, it has been suggested that one could differentiate eurytopic species such as Talitroides alluaudi from more stenotopic species such as Arcitalitrus dorrieni on the basis of physiology alone (Bousfield, 1982, 1984). The paucity of relevant physiological studies (particularly with regard to the more terrestrial amphipods, lamented both by Hurley (1959, 1968) and Laso-Wazem (1984), however, has so far rendered even a qualitative assessment of these differences impossible.

The preceding four chapters have gone some way to redressing our lack of detailed knowledge of even the most fundamental aspects of talitrid physiology. The present chapter seeks to combine these new data with a critical reinterpretation of the relevant information available from the literature, in order to review the state of our

knowledge and to provide a framework for subsequent incisive investigation. (Note: no attempt has been made to include data on the reproductive and developmental biology of these animals, as this has been very adequately reviewed recently (Morino, 1978; Wildish, 1979, 1982b; Friend and Richardson, 1986). Similarly, the following account does not purport to replace, but to complement, the reviews which exist at present (Hurley, 1959, 1968; Powers and Bliss, 1983; Little, 1983; Friend and Richardson, 1986)).

The recent work of Bousfield (1984), sub-dividing the Talitridae into four eco-morphological though phyletically overlapping groups (outlined in chapter 1) has made possible more meaningful intra- and interspecific physiological comparisons. It should now be possible to interpret particular aspects of talitrid physiology not solely in the context of their immediate habitat, but also with reference to their evolutionary background.

As there is no information at present on palustral amphipods (Group I), they have been omitted from the consideration. Even though distinct groups can be designated, there is a great deal of convergent evolution between them. Interpretation of mechanisms (as of structures) must thus be careful and cautious. Equally, one should never imply that mechanisms present in living species evolved directly from those of other living species. Comparative studies of extant species are, however the only handle on reality that we can grasp in a group bereft of fossil representatives, so it is important that functional studies go hand in hand with phylogenetic speculations to test their feasibility. In Bousfield's (1984) scheme there are two major phylogenetically different groups of terrestrial talitrids, both of which are referred to as landhoppers (Group IV) . Group IV:2 consists of sexually dimorphic species descended from beachflea (Bousfield's, Group II) progenitors. The second more ancient group

(Group IV:1) is derived directly from littoral ancestors. Powers and Bliss (1983) suggested that the combination of presumed terrestrial/ marine/ littoral traits probably reflected the independent paths of evolution followed by different terrestrial species. It is hoped that an assessment of the physiology of these different groups may help demonstrate the primary adaptations for terrestrial evolution.

7.1.2 Definition of the term "terrestrial"

Hurley (1959, 1968) defined "terrestrial" in the context of his studies on the Talitridae as "being independent of the sea or standing bodies of water for breeding, feeding and distribution." Accordingly, he separated the supralittoral species from what he regarded as fully terrestrial species to prevent misuse of the term as applied to such supralittoral species as Talitrus saltator (Dahl, 1946) or Orchestia gammarellus (Williamson, 1951a, see also Barnard, 1969). In these supralittoral animals, dispersal is passive and takes place in sea water (Karlbrink, 1969). This definition is in full agreement with that suggested by Powers and Bliss (1983). Such a definition, however, is not without problems. Although the common beachflea of British shores, O. gammarellus, is generally regarded as supralittoral in its distribution, in N.W. Scotland and towards its southern geographical limit, it can be found many metres above sea-level (see chapter 2). At these distances from the shore, dispersal is presumably no longer facilitated by the sea although it is entirely possible that it could involve standing bodies of water. Therefore, in some locations, this animal is semi-terrestrial whereas in others it must by definition be regarded as terrestrial.

Hurley's (1968) definition thus has its limitations which become more acute if we press for quantitative differences in biology between semi- and fully terrestrial species. Fortunately, this does not

represent much of a problem within the confines of the present study. As regards physiology, the differences between what would commonly be regarded as semi- and euterrestrial species are largely differences in degree rather than kind. We can still hold to the definitions given by Hurley (1959, 1968) and Powers and Bliss (1983) providing we keep these limitations in mind.

7.2 WATER RELATIONS

7.2.1 Desiccation

In the air, conditions which favour oxygen uptake will also favour water loss. Those arthropods which have become fully adapted to land, exhibit a number of adaptations which retard water loss, eg. the presence of an impermeable cuticle and the internalisation of the respiratory organs (tracheae in insects) (Little, 1983). It has long been recognised, however, that both semi- and fully terrestrial amphipods are very susceptible to desiccation stress (Chevreux, 1888; De Geurne, 1888; Hurley, 1968). The aquatic amphipods, Gammarus duebeni and G. oceanicus were able to survive a maximum of 228 and 120 h respectively, in fully saturated air at 19^{0}C (Lagerspetz, 1963). Members of the family Talitridae, however, were able to survive indefinite exposure to aerial conditions as long as 100% relative humidity (R.H.) was maintained. Platzman (1960), despite the highly artificial nature of her experimental conditions, also showed that survival at 26.5^{0}C of the two beachfleas, Talorchestia megalophthalma (44 mins) and Orchestia agilis (33 mins) was greater than the fully marine Amphithoe sp. (13.5 mins) in dry air (0 % R.H.). She concluded that this increase in survival at low R.H. was an adaptation to terrestrial life.

Williamson (1951a), examining the survival times of three semi-terrestrial talitrid species in air of different humidity, found that Talitrus saltator always survived considerably longer than both Talorchestia deshayesii and Orchestia gammarellus. At R.H. 95% (25^{0}C) he found that survival time for Talitrus was 16 h which was almost twice that of Orchestia or Talorchestia (9 h). Williamson (1951a) noted that the survival times measured for O. gammarellus at different humidities by Backlund (1945) were greater than those found in his own studies; this he attributed to the different methods of humidity control used. It should be noted, however, that not only were the experimental techniques and protocol different in the two studies, but that in both cases, the weight range of the individuals examined was not clearly specified. In a recent publication, Moore and Francis (1985a) confirmed that O. gammarellus was extremely sensitive to desiccation although not quite as sensitive perhaps as Williamson's data suggest; at 23^{0}C and R.H. = 82.8%, 88% mortality was recorded after 10 h. Pallaud (1954) found, during an ecological study of T. saltator, that at 84% R.H. (20^{0}C) this species survived for 19 h. Unfortunately, he too was not specific about the weight range of experimental animals which, coupled with the difference in experimental method, makes a comparison with Williamson's data difficult. Bowers (1964), during a comparative study of two beachfleas, Orchestoidea californiana and O. corniculata found that there was no difference in humidity tolerance between the two species in "dry" air. He recognised, however, that the artificially low humidities induced were too low to allow variations in tolerance to be detected. He found that around 25^{0}C, individuals of both species succumbed in less than 4 hours in "dry" air. Doubtless the fumes from the sulphuric acid, used as a desiccant, hastened the demise of his experimental animals.

Recently there have been two studies published concerning the humidity tolerance of some fully terrestrial leafhopper species. Lazo-Wasem (1984) found that in the synanthropic Arcitalitrus sylvaticus, humidity tolerance was apparently lower than that found for O. gammarellus, Talorchestia deshayesii or Talitrus saltator. The euterrestrial Mysicotalitrus (=Talitrus) tasmaniae displayed a greater tolerance of desiccation stress than Keratroides (=Talitrus) vulgaris or another undescribed Mysticotalitrus sp. (Richardson and Devitt, 1984). At 95% R.H. (15^0C), the time taken for 50% of the animals (10 animals initially) to die was 2.6, 2.1 and 1.9 days respectively. There was little variation, however, between the three species below 95% R.H. (ie 93, 90, 85 & 80 % R.H.). Richardson and Devitt (1984) suggested a likely correlation between the more hardy nature of M. tasmaniae when compared with K. vulgaris, in that the former is able to colonise warmer and less humid environments. Despite the basic and somewhat limited nature of the experiments, this study clearly demonstrates (i) the value of examining the humidity response of animals to a finer gradation (and more realistic range) of relative humidities than has hitherto been examined, (ii) that there are degrees of desiccation resistance in landhopper species, ie. some species are better adapted for drier habitats than others (see Wildish, 1979), and (iii) the difficulties involved in comparing data from different studies which may differ in their methodology and in the presentation of the data. This is clearly shown when an attempt is made to compare the results for these particular landhopper species with A. sylvaticus. It is apparent that, apart from broad generalisations, there is no great value in such comparisons at present.

It may at first seem surprising that desiccation tolerance should be greater in supra-littoral than euterrestrial genera. This will be considered more fully below.

7.2.2 Transpiration rates

Transpiration rates and percentage body weight loss in response to low humidity have been measured in O. gammarellus (Williamson, 1951a; Moore and Francis, 1985a), T. saltator (Williamson, 1951a; Pallaualt, 1954), T. deshayesii (Williamson, 1951a) and A. sylvaticus (Lazo-Wasem, 1984), and typically the values were high. As mentioned above, any attempt at a detailed interspecific comparison is rendered unprofitable by the multiplicity of differing experimental variables between investigations. It is clear, however, that smaller animals loose water faster than larger animals. It would also appear that in O. gammarellus (and in all probability all of the talitrids yet studied) that the rate of evaporation from the animal's body is not controlled: Moore and Francis (1985a) found that dead animals lost water at an equivalent rate to live animals. This fact alone may explain the observed differences in humidity tolerance and transpiration rates of the landhopper and beach/sand flea genera. The landhoppers have a typically smaller body size range (hence larger surface area) than the predominantly heavy-bodied supralittoral animals. Body water loss greater than 25% resulted in the death of O. gammarellus which, allowing for differences in pre-experimental water loss, is similar to figures obtained from some terrestrial crabs (Moore and Francis, 1985a).

7.2.3 Sites of water loss

The majority of land arthropods possess an impermeable cuticle and, in the case of the insects, breathe though a system of air tubes called tracheae. Beachfleas and landhoppers, however, apparently lose

water from the whole body surface. This has been demonstrated semi-quantitatively by silver staining techniques in O. cavimana (Graf and Magniez, 1979), O. gammarellus (Moore and Francis, 1985a), Transorchestia chiliensis and Chroestia lota (Marsden, 1984, 1985) and A. dorrieni (J.I.S. Unpubl. obs.). Individuals of O. gammarellus in which the body surface had been smeered with vaseline, lost water at a slower rate than untreated control animals (Moore and Francis, 1985a).

Semi- and fully terrestrial woodlice have a well-defined layer of wax covering the cuticle (Hamilton et al., 1975) which is presumed to be an adaptation to improve water retention. Although offerring no suggestion as to their function, Shyamasundari and Hanumanitha Rao (1974) have noted the occurrence of mucous glands on the first and second antennae, peraeopods and uropods of the semi-terrestrial species, Talorchestia martensii and of Platorchestia platensis. They also noted that none of the mouthparts, gnathopods or pleopods possessed such glands but unfortunately they do not give any indication as to the stain retention (Alcian blue + safranin) of the general body surface. It has recently been noted that there is a thin layer of fluid present on the body surface of landhopper species, which produces a strong iridescence (Duncan, 1985a; see also Moore and Spicer, 1986).

Moore and Francis (1985a) have demonstrated the presence of an epicuticular wax layer in O. gammarellus which may be secreted by a system of pores in the integument (Sellem and Graf, 1982; Moore and Francis, 1985a). These pore types and their spatial arrangement on the cuticle surface are similar in both beachflea (Group II) (Moore and Francis, 1985a) and landhopper (Group IV) species (Duncan, 1985a). The surface wax of O. gammarellus was found to have a melting point (36-44^{0}C) similar to that reported for the terrestrial oniscoidean isopod

Oniscus asellus (37^0C), a feature which may restrict the activity of both animals, particularly with regard to exposure to full sunlight.

Unfortunately, the relative importance of different potential sites of water loss in any talitrid species has not yet been properly quantified. The gills of O. gammarellus, O. cavimana and A. dorrieni show substantial stain retention (Graf and Magniez, 1969; Moore and Francis, 1985a; J.I.S. unpubl. obs.) Presumably this is also the case in Transorchestia chiliensis, although Marsden (1985) only mentions that the pleopods, telson and the basal segments of the second antennae are the most permeable areas of the body. The pleopods of O. cavimana are also implicated as sites of water loss (Graf and Magniez, 1969) although Moore and Francis (1985a) did not mention them in the results of their silver staining of O. gammarellus. The staining of the epimeral and coxal plates is confirmed for O. cavimana and A. dorrieni. These were stained the least of stained surfaces in O. gammarellus by Moore and Francis (1985a).

All the talitrids examined in chapter 4 (with the exception of A. dorrieni) had gills with significantly smaller weight-specific gill areas than the marine amphipods examined by Moore and Taylor (1984). Within examined talitrids, the gill area per unit weight declined in an order which can be roughly correlated with desiccation resistance for each species. Somewhat paradoxically, however, the animal with the greatest overall gill area was also the most terrestrial of the species studied. This strongly suggests that reduction in gill area may be regarded as an adaptation to reduce permeability (Taylor, 1985) and that gill reduction per se is not a pre-requisite for talitrid amphipods assuming a fully terrestrial existence. It could also be suggested that pleopod reduction may be related to or, at very least, have consequences for water conservation, since it has been suggested that the pleopods are an important site of water loss (Marsden,

1985) - although this still requires experimental verification.

Finally, it should be noted that there may be a relationship between grooming behaviour (see chapter 2) and water loss. In terrestrial amphipods, fine granular material may abrade articular membranes promoting desiccation (B.D. Valentine, in Holmquist, 1982). In this way, grooming in terrestrial amphipods may reduce the incidence of damage and therefore reduce the likelihood of desiccation.

7.2.4 Water loss as related to habitat and orientation

As the result of their susceptibility to desiccation, the Talitridae are confined to a largely cryptozoic existence and are much more limited in their adaptive radiation than other terrestrial arthropods such as insects and, to a lesser extent, woodlice.

It is known that O. gammarellus inhabits an almost fully saturated (>95% R.H.) environment in the supra-littoral zone (Backlund, 1945; Moore and Francis, 1985a). The few qualitative data that exist, would tend to suggest that this is also true of the euterrestrial amphipods (see Hurley, 1968 and Friend and Richardson, 1986). To maintain their presence in this saturated environment, talitrid amphipods are greatly dependent on a number of inter-related behavioural mechanisms. The aggregation of large numbers of O. gammarellus in the field has been observed (J.I.S. Unpubl. obs.) often involving individual animals being in close bodily contact with one another. In the landhoppers O. hurleyi and O. patersoni, however, these animals actually avoid physical contact (except for mating) with each other (Duncan, 1969). Duncan also observed that one individual may even repel another by lying on its side and 'kicking' with its abdomen.

Talitrids are primarily nocturnal in their habits and many of the supra-littoral species exhibit a diurnal rhythm of activity (see chapter 2). Orchestia gammarellus is known to show a preference for the highest humidity available (Backlund, 1945; Williamson, 1951a). This animal will move more quickly, hopping and jumping often in dry air (Williamson, 1951a). Talitrus saltator has been shown to burrow to a depth at which the moisture is optimum (Williams, 1983b). Burrowing itself is thus an important water conservation measure.

In an attempt to locate structures that could be referred to as humidity receptors, Williamson (1951a) carried out a number of strategic amputations on three talitrid species he was studying. The first and second antennae received particular attention, but with no success. He also attempted to cover different parts of the body surface with wax but this resulted in the untimely demise of the experimental animals. Williamson concluded that the Talitridae had no specific hygrometer-like receptors but reacted instead to different amounts of water loss. Williams (1983a) rightly pointed out that Williamson (1951a) omitted to clarify his use of the term water loss which could be understood either as the amount of water loss per unit time (which is a function of the saturation deficit [S.D.]) or as water loss as a percentage of the initial body weight (this is a function of both exposure time and S.D.). In his own experiments involving T. saltator, Williams (1983b) found that when he kept the S.D. constant, the animal was still able to orientate, indicating that it was reacting to water loss as a percentage of the initial body weight.

As the effects of light, temperature and humidity are inter-related, it is not altogether surprising to find that these animals are able to orientate with respect to each of these features, eg. the degree of pre-experimental water loss has been implicated in

"resetting" the orientation pattern of particular supra-littoral species (see p 23 chapter 2).

The water requirements of many arthropods are satisfied by water taken in with the food supplemented by drinking free water (Berridge, 1970). It has been shown that O. gammarellus is able to replace lost body water from both a fully saturated atmosphere and from standing bodies of water (Moore and Francis, 1985a). The water loss sustained during acute desiccation stress would appear to be at the expense of the haemolymph (Moore and Francis, 1985a; J.I.S., unpubl. obs.). The 'blueing' of the limbs noted by Rawlinson (1937), Williamson (1951a) and Moore and Francis (1985a) may be due to chromatoprotein pigments in the haemolymph becoming fibrillar after water removal.

Terrestrial amphipods also carry exosomatic water in their brood pouch (Hurley, 1968; J.I.S., unpubl. obs.). Duncan (in Hurley, 1968) has assumed that dew is sufficient to provide the quantity found. Therefore, there may be no need for the amphipod to search for standing bodies of water.

7.2.5 Oostegites and desiccation resistance of eggs

It has been suggested that the oostegites of Austrotroides crenatus, which are broader than those of similar Austrotroides spp., were developed in response to the climate of the Adelaide (Australia) area which is much drier than the forests of Western Australia (Friend, 1982). In this connection, it is interesting that the fully developed oostegites of the landhopper A. dorrieni have a much smaller surface area than those taken from Orchestia gammarellus or O. mediterranea (Moore and Spicer, 1986; J.I.S. Unpubl. obs.). This may be related to the occurrence of desiccatory stress in their respective habitats; O. gammarellus is subject to greater extremes of humidity variation in the supra-littoral zone than A. dorrieni which inhabits

the relatively stable (perhaps) saturated environment of the leaf litter.

7.2.6 Conclusions

It is evident that our knowledge of the effects of humidity on talitrid amphipods is grossly deficient. As noted above, much existing work is of very little comparative value. It is clear that talitrids in general are extremely susceptible to desiccation, far more so than other terrestrial arthropod groups, but choice experiments involving more realistic gradations of humidity need to be performed, particularly with regard to the leaf litter inhabitants. It is suggested for the future, that experimental techniques and animal body size be standardised as far as possible and that a number of different species be examined simultaneously, under identical conditions.

7.3 IONIC AND OSMOTIC REGULATION

7.3.1 Introduction

The ability to maintain a suitable internal milieu in the face of osmotic stress has played a most important role in animal evolution for two main reasons. First, animals are restricted in their geographical distribution by environmental factors, one of the most important of which is the osmotic nature of the environment. Second, geographical dispersal followed by genetic isolation is an important mechanism for the evolutionary divergence of species. It could be argued that the arthropods, and in particular the insects, would have been less successful in their invasion of fresh-water and land if they had not evolved a means of regulating their extracellular ionic milieu. It has been suggested recently that physiological adaptations

to retain ions in the body have been more important in the evolution of the landhoppers than adaptations to resist desiccation (Friend and Richardson, 1986). This theory is supported by the fact that, as we have seen, humidity tolerance appears to be better developed in the beachflea genera than the landhoppers, although it must be kept in mind that there is as yet no conclusive evidence for such a difference existing between animals of equivalent weight.

7.3.2 Osmoregulation

Talitrid amphipods have colonised both the estuarine and fresh-water environments and so highly developed powers of osmotic regulation may be anticipated (see Bousfield, 1958 in his list of terrestrial adaptations). Yet despite this, very little pertinent data exist in the literature. Indeed with the exception of the data presented in chapter 6 and a few isolated values scattered thoughout the literature, (Bock, 1967; Little, 1983; Moloney and Nicolson, 1984), the only papers to deal with talitrid (beachflea only) osmoregulation are those of Bock (1967) and Moore and Francis (1985a). (Note: Further studies are at present being carried out on talitrid osmoregulation, including a fully terrestrial species at Bristol university; Dr. C. Little, pers. comm.).

Both *Orchestia gammarellus* and *Platorchestia (=Orchestia) platensis* hyper-regulate when fed either with food of a reduced salinity or when immersed in dilute media and hyporegulate under conditions of hypersaline exposure. A similar ability is found in fully marine amphipods such as *Gammarus duebeni* and *G. oceanicus*. Both of these features have been regarded as pre-adaptations to terrestrial existance in other crustacean groups (Mantel and Farmer, 1983). It should be noted, however, that in *O. gammarellus*, this ability is apparently less efficient when the animal is immersed (Moore and Francis, 1985a; see also Price and Holdrich, 1980). This

may be due to the inappropriate nature of this medium for O. gammarellus.

While the relative HCY content of amphipod haemolymph becomes more stable with the move onto land, the level and range of absolute HCY content may be unaffected by the animals' ecological position on the shore (Wieser, 1965). The stability of the relative HCY concentration in Orchestia may be indicative of a strict control mechanism perhaps related to the role of this molecule in osmoregulation.

Two different types of relationship between blood protein and blood osmolarity have been found from examination of a number of marine and semi-terrestrial Crustacea (Pequeux et al., 1979). In aquatic euryhaline species including Carcinus maenas, there is an inverse relationship between blood protein and blood osmolarity, for reasons similar to those oulined above, but in the semi-terrestrial species represented by Uca minax and Ocypode quadratus, the relationship is direct. Pequeux et al. (1979) postulated that the reason for this coupling of high blood protein with high blood osmolarity may relate to the maintenance of colloidal osmotic pressure in the body fluids (but see Mangum and Johansen, 1975.) in animals continually confronted with problems of water availability.

Despite the semi-terrestrial habit of O. gammarellus, it is clear that this species is more closely allied to the aquatic euryhaline decapods in its method of blood and cell volume regulation, than to the semi-terrestrial species examined by Pequeux et al. (1979). This feature may be indicative of the generally water-saturated environment inhabited by even the most terrestrial of the talitrid genera. Under such conditions, water loss (perhaps surprisingly) is not to be regarded as the pre-eminent barrier to

talitrid amphipods colonisation of land (see above). Individuals occurring above the high water mark (AHW animals) have a blood and tissue composition quantitatively (if not qualitatively) different from their BHW counterparts. They are characterised by a low total FAA and a low ionic content (producing a low osmotic pressure), together with a high blood protein concentration (as opposed to high total FAA, high osmolarity and low protein content which is characteristic of most BHW animals). Presumably as this situation is fully reversible, the difference is environmentally induced and a genetic component is unlikely. This does not, however, alter the fact that the oxygen transporting properties of the blood of the two groups are different.

In the Azores, O. gammarellus is found far inland and has possibly 'thrown-off' a fully terrestrial species (Hurley, 1968), O. chevreuxi (Andersson, 1961). A similar case exists with two populations of P. platensis (Tamura and Koeski, 1974) where morphologically identical animals inhabit both sea-shore and local forest environments (although it is now considered that these are distinct species. They are certainly separated by their reproductive behaviour. It could be expected that O. chevreuxi and O. platensis japonicus would exhibit blood /tissue characteristics similar to AHW O. gammarellus. Consequently, genetic isolation of these species from their sea-shore progenitors will result in a divergence in their blood/tissue characteristics with the resultant consequences for the transport of oxygen in the blood (see below). It would, therefore, be of great interest to compare the ionic and osmotic regulatory ability and mechanisms of a closely related pair of species such as O. gammarellus and O. chevreuxi. Similarly, further comparison of the beachflea species with some true landhopper species may shed some light on the interspecific differences in physiology in these two groups.

Table 7.1 Values for the osmotic pressure of talitrid amphipod haemolymph.

Species	O.P. (mOsm)	Reference
Orchestia gammarellus	828	Moore and Francis (1985a)
Orchestia cavimana	360	J.I.S., unpublished
Platorchestia platensis	806	Bock (1967)
Talorchestia capensis	836	Moloney and Nicolson (1984)
Unidentified eu-terrestrial talitrid	400	Little (1983)

Table 7.1 contains all known values for the osmotic pressure of talitrid amphipod haemolymph. These values are all comparatively high, including O. cavimana and the leaf-hopper species. This is often regarded as evidence for transition onto land via the littoral zone rather than a route involving fresh-water. However, this is not by any means certain since O. cavimana is a fresh-water talitrid which has been found far inland (Dorsman, 1935). It may be, while landhoppers have a littoral origin, that the more terrestrial beachflea species may have colonised land via both fresh-water and supra-littoral marine routes. Having data on only two beachflea species though, it is impossible to assess the ecological significance of any intergeneric differences that may exist.

7.3.3 Ionic regulation

It has been suggested that the maintenance of chloride ion concentration is responsible for limiting the penetration of terrestrial beachfleas to oceanic islands or maritime habitats (Bousfield, 1968). A recent study, however, has provided some evidence that the critical ion species may not be chloride but its associated cation sodium (Moore and Francis, 1986a). Therefore, it is not surprising that this ion is so well regulated by O. gammarellus (chapter 6). Orchestia gammarellus can tolerate a concentration of sodium ions three orders of magnitude below that of seawater. This amphipod, however, could not exist on natural or enhanced concentrations of calcium or potassium (chlorides) alone when sodium ions were removed (Moore and Francis, 1986a). The suggestion that the difference between beachfleas and landhoppers lies in their differing ionic regulatory abilities has yet to be substantiated. The attraction

of this theory, comes mainly from the rejection of desiccation tolerance as a major criterion for land life and no more likely explainations have been put farward. The theory has one disadvantage in that it presupposes that no beachflea species has evolved via a fresh-water route.

Although marine decapods store some calcium, they lose a large proportion of their total body calcium when they moult (Passano, 1960). This is regained by active reabsorption from the surrounding seawater (Robertson, 1960). Semi-terrestrial amphipods no longer have unlimited access to the sea. In consequence, calcium storage is high eg. in Orchestia (Graf, 1965, 1974; cf. also Graf, 1966, 1967, 1971, 1972) and in other terrestrial Crustacea (Sparkes and Greenaway, 1984). It is known that calcium levels in the haemolymph fluctuate in response to salinity changes (chapter 5) as well as during different stages of the moult cycle in O. gammarellus (Graf, 1971). During the pre-exuvial period, calcium reabsorbed from the old cuticle is stored in the form of calcareous concretions in the posterior caeca of the midgut. In the post-exuvial period such stored concretions are extruded into the haemolymph and reabsorbed within one or two days to mineralise the new cuticle (Meyran et al., 1984). Presumably a similar process occurs in landhoppers, in which it should be of even greater importance (see below).

Reid (1947) noted that while most amphipods harden their exoskeleton by calcium carbonate deposition, this did not appear to be a feature of the Talitridae. He suggested that, rather than this "physiological peculiarity" being related to lightening the weight of a saltatory animal, the "absence of such a stiffening material might help to make terrestrial existence easier in that a supple animal is better fitted for crawling into dry apertures than is a rigid one."

Such softening of the exoskeleton, however, may be confined to

the beachflea and sandhopper genera. Haswell (1882), describing Talorchestia marmorata (which despite its generic attribution belongs to Bousfield's group IV:1, the landhoppers) noted that the whole integument was very hard. Duncan (1985a) has also noted that the cuticle of landhoppers is well supported by calcium compounds. Personal observations on Arcitalitrus dorrieni confirm that the appearance and texture of the integument is more closely related to that of marine amphipods than to the supra-littoral beachfleas referred to by Reid (1947). This would seem to suggest that selective pressure leading towards suppleness of the integument in the beachflea and sandflea groups was not present in the case of the most ancient landhopper group. What is not in doubt, however, is that an important physiological difference exists between the two groups as regards the mechanism of calcification. Comparative studies on this topic deserve high priority in the future. Wieser (1967) has outlined the problem of copper availability and storage in Crustacea that no longer have recourse to the sea. It is similar in nature to that outlined above for calcium. The copper content of the hepatopancreas of Orchestia is elevated and less variable than in a number of aquatic amphipod species (Wieser, 1965). This was also observed in the woodlice, which have been more thoroughly studied than the Talitridae, where such high copper concentrations are related to the need to conserve ions that has resulted from the move onto land (Little, 1983). In the terrestrial isopods, copper is obtained from their decaying leaf litter food (Coughtrey et al., 1980); the efficiency of copper assimilation varies inversely with the moisture content and the state of preservation (undecayed) of the leaf litter (Wieser, 1968). A similar situation may occur in the Talitridae but, as yet, this is still subject to investigation.

Fully terrestrial amphipods are subjected to lower environmental pH conditions than are their aquatic (marine) counterparts (Friend and Richardson, 1977). Some recent work on the semi-terrestrial O. gammarellus (Moore and Francis, 1986a) has shown a marked degree of pH tolerance in this species although sub-lethal pH values may interfere with vital processes like feeding (Moore and Francis, 1985b). Such physiological tolerances may be pre-adaptive for the fully terrestrial environment.

7.4 NITROGENOUS EXCRETION

An important difference between terrestrial arthropods from a variety of groups is the nature of their nitrogenous end products. Crabs, woodlice and myriapods excrete nitrogen mainly as ammonia whereas arachnids and insects excrete insoluble purines, guanine and uric acid the last of which reduces water lost during excretion (Little, 1983). Dresel and Moyle (1950) found that O. gammarellus had adapted to a terrestrial life while retaining a primitive ammonotelic type of metabolism. The suppression of nitrogen metabolism in terrestrial isopods and amphipods as they suggested, however, has been shown to be wrong in the case of the former (Wieser et al., 1969). It may be that this is also true for the amphipods.

Little (1983) suggested that the apparent reduction in excreted nitrogen probably reflected the use of gaseous ammonia as an end product. In the Talitridae the antennal gland has been lost (or at least is greatly reduced) and it is thought that excretion takes place via the digestive tract (Bousfield, pers comm. in Kaestner, 1970). This would also appear to be true for euterrestrial landhopper species (See Morton, in Friend and Richardson, 1986). Friend and Richardson (1986) have commented rightly that the retention of an ammoniotelic

nitrogenous excretion is not surprising considering the moist habitat of the Talitridae. It is also of interest in this connection that the semi-terrestrial O. gammarellus shows greater tolerance to increased ammonia concentrations than does the fresh-water Gammarus pulex (Moore and Francis, 1986a).

The uric acid (a possible alternative waste product) content of whole animals was similar in both semi-terrestrial (Orchestia sp. 0.08 $mg.g^{-1}$ wet wt.) and marine (Marinogammarus spp. 0.07 - 0.10 $mg.g^{-1}$ wet wt.) amphipods (Dresel and Moyle, 1950).

It is clear that our knowledge of nitrogenous excretion in talitrid amphipods is grossly deficient. From the little evidence currently available, it could be suggested that there has been no change in the method of nitrogenous excretion accompanying the transition from sea to land in amphipod Crustacea. This retention of an ammoniotelic nitrogenous excretion may be associated with the moist character of talitrid habitats. Equally, it may highlight our ignorance of the basic processes involved and our reliance on extrapolation from other groups.

7.5 RESPIRATION

7.5.1 Aerial:Aquatic respiration

It would appear, in both the beachflea and landhopper species examined, that as a species becomes less dependent on standing bodies of water for its survival, it loses the ability to maintain an aerial rate of oxygen consumption when submerged (see Table 3.4 in chapter 3). In many instances, however, this does not result in the death of the animal as long as the stress of immersion is not compounded by other extraneous factors (see chapter 3. also Bowers, 1964). For example, none of the animals examined in this present study survived

immersion at temperatures in excess of 20^0C. It is difficult to assess the relationship between the loss of bimodal respiration and the reduction of gill area in the beachflea and landhopper groups studied. Indeed without profound changes in the nature and/or site(s) of gas exchange it is difficult to offer a satisfactory explanation for the suppression of aquatic respiration observed (see below). Arcitalitrus dorrieni (Group IV:1) and other fully terrestrial landhoppers (Makawe (Orchestia) hurleyi and Talorchestia (Orchestia) patersoni [Group IV:2]; K.W. Duncan, pers. comm.) are incapable of prolonged aquatic immersion regardless of exposure temperature. They have completely lost the capacity to respire under water with initial aerial:aquatic oxygen consumption ratios in the region of 2 - 3, despite the retention of an aquatic gill area. It is unclear, however, whether respiratory or osmotic stress was the primary cause of death (Duncan, 1969; J.I.S. unpubl. obs.). In the case of the South African landhopper (group IV:1) Talitriator eastwoodae (Methuen, 1913), however, submersion for up to 10 days in ordinary tap water seems to have no ill effect (Lawrence, 1953). It is clear that there is a greater need for quantitative data on the survival and oxygen consumption in air and water of various landhopper species (both IV 1 and 2) before the overall picture becomes clear.

Although there was not the expected increase in metabolism sometimes associated with the ready availability of oxygen in the aerial environment (Edney, 1960) both beachflea and landhopper representatives examined in chapter 3 were still able to maintain a constant aerial oxygen consumption at 30^0C for a number of hours after initial exposure. In contrast, the intertidal amphipods E. obtusatus and E. pirloti, sustained aerial oxygen consumption was not possible at temperatures in excess of 250_C. This is clearly an adaptation to

the increase in temperature fluctuation experienced in the terrestrial habitat as are the low Q_{10} values for beachflea and landhopper oxygen consumption rates.

The relative antiquity of terrestrial species from both talitrid groups may be shown in their response to immersion. In the semi- and almost fully terrestrial beachfleas, there are examples of retention of bimodal respiration, (although aquatic rates of oxygen consumption are considerably reduced when compared with eulittoral or semi-terrestrial beachflea species eg. O. cavimana) but there is also loss of bimodal respiration in amphipods derived from beachflea progenitors, eg. Makawe hurleyi and Talorchestia patersoni. In the beachfleas, retention of bimodal respiration, the conservation of pleopods and the rhythmic response of these structures upon immersion, may be indicative of an incomplete adaptation of these animals to an aerial existence (see also Walshe-Maetz, 1952, 1956 on the lack of respiratory regulation during hypoxia). This is in sharp contrast to the landhopper species in which there is a reduction or total loss of pleopods and animals, while initially having a high aerial: aquatic O_2 consumption rate, are unable to survive prolonged immersion.

7.5.2 Oxygen transport

The only information on the oxygen transporting properties of talitrid haemolymph is that presented herein. The semi-terrestrial beachfleas O. gammarellus and O. mediterranea both have a high affinity pigment with a moderate Bohr shift. As previously mentioned (chapter 5), this does not appear to support the hypothesis that a low oxygen affinity is an adaptation to living on land in decapod crustaceans (Young, 1972). However, the properties of Orchestia haemolymph may still be related to the animals' move onto land. As noted above, one of the major problems facing animals coming out onto

land via the littoral zone, must be desiccation of the surfaces of respiratory gas exchange. In association with this it is known that O. gammarellus and O. mediterranea (among others) show a reduction in their total gill area which may be related to the need to conserve water. Whatever the reasons for the reduction, it has certain consequences for oxygen transportion: it will reduce the overall rate of oxygen diffusion into the blood. This presupposes 1) that the gills are (though not necessarily the only), the main site of gas exchange and 2) that with the reduction in gill area, the gill cuticle either remained the same thickness or become thicker, and 3) that there is no net change in blood flow. As the rate of diffusion across the gills is directly proportional to the partial pressure gradient, the greater the oxygen affinity of the pigment, the faster will oxygen be taken up into the blood. It is often the case that the post-branchial blood Po_2 in decapods is low (Redmond, 1968) which suggests that the respiratory consequences of water conservation may outweigh those of enhanced oxygen availability in air.

A high affinity pigment is often regarded as an adaptation to hypoxia tolerance (low external Po_2 as opposed to low internal Po_2 considered above) but because of the high diffusion rate of oxygen in air, terrestrial micro-habitats are not normally liable to oxygen depletion. In soils, however, although oxygen tensions of around 150 Torr are found even at depths of 30 cm, heavy rain may seal interstices at the surface preventing free diffusive exchange. In such a case, the oxygen tension can fall rapidly to very low levels, due to the activity of soil organisms, and be accompanied by a corresponding increase in the partial pressure of CO_2. In these circumstances it may be several days before optimal conditions are restored (Jones, 1972). Therefore, the presence of such a high affinity pigment may appear advantageous to a cryptozoic leaf-litter inhabitant such as A.

dorrieni. Duncan (1969), however, has noted that M. hurleyi will climb upwards when the soil is waterlogged whereas T. patersoni which are not known to climb may frequently be found dead in small pools after heavy rain. Moore and Spicer (1986) also noticed that Arcitalirus dorrieni appeared to shun waterlogged sites too.

From the work carried out on O. gammarellus, it appears that when beachflea species 'throws off' a terrestrial species, the euterrestrial novelty (equivalent to AHW animals?) may have different in vivo oxygen carrying properties of the blood from its supralittoral progenitor (equivalent to BHW animals). Until the oxygen transporting properties of such species as O. cavimana, Platorchestia platensis japonicus and P. platensis platensis are examined and the results compared with those of the present study, such suggestions must remain highly speculative. Nevertheless even speculations may be helpful in making predictions about ecologically different species and may throw some light upon the relationship of the gas exchange structures to the oxygen transportation system.

It is suggested, therefore, that as the oxygen affinity of the rspiratory pigment of O. gammarellus (AHW) increases relative to that of O. gammarellus (BHW) due to the reduction in influence of divalent ions and a decrease of in vivo pH, so a similar situation may exist in the two subpopulations of P. platensis studied by Tamura and Koseki (1974). They found that in spite of morphological uniformity, the supralittoral and forest populations of P. platensis showed two distinct non-breeding periods and over-winter in distinctly separated developmental stages. This was undoubtedly accompanied by a reduction in the total haemolymph ionic composition of the forest dwelling P. platensis japonicus (equivalent to AHW animals ?) compared with the supralittoral P. platensis platensis (equivalent to BHW animals ?). We

could also postulate on the same grounds, the likely presence of a low oxygen affinity pigment in the haemolymph of O. cavimana (a close relative of O. mediterranea).

Indeed such a feature may help explain the low aerial:aquatic oxygen consumption ratio in O. cavimana (cf. O. gammarellus and O. mediterranea). It is suggested that the high affinity pigment of O. gammarellus and O. mediterranea is a relic of their intertidal history. For their littoral progenitors, both hypoxia stress and reduction of gill area in response to desiccation stress would make the possession of a high affinity pigment desirable. With the emergence onto land, a high affinity pigment would be redundant except perhaps for the short periods of accidental inundation occassionally experienced by these animals. It is known that the eulittoral O. mediterranea lacks any great measure of respiratory independence during hypoxia; the pleopod beat is quite insensitive to changes in ambient Po_2 (Walshe-Maetz, 1952, 1956). This, together with the retention of bimodal respiration (see above), could be regarded as evidence of its incomplete adaptation to an aerial environment. Certainly the high P_c value found for O. mediterranea would appear to be inconsistent with the presence of a high affinity pigment (Herreid, 1980). Since there is apparently no selection pressure for retention of a high affinity pigment, with landwards progression (due to the corresponding decrease in the ionic composition and the osmotic pressure of the haemolymph), pigment affinity decreases. Such a theory would predict a low oxygen affinity for euterrestrial beachflea and landhopper species.

The temperature insensitivity of the respiratory pigment may be taken, like the low Q_{10} values, as an adaptation to the increased thermal instability of the littoral zone (see chapter 5 for details), and would be pre-adaptive for terrestrial life.

7.6 SENSORY APPARATUS

Associated with the colonisation of land, there has been a marked reduction in the first antennae in Talitrus, and as first noted by Bate (1862), the olfactory lobes of the brain become reduced. Therefore any increase in the sensory function of the second antennae may be associated with greater development of the tritocerebrum. Madsen (1960) found that the development of glomeruli cells together with neuropiles in the tritocerebrum of Orchestia greatly exceed that of Gammarus. The second antennae are also used as prehensile structures during copulation (in the absence of prehensile first gnathopods), so they have an important tactile role in addition to olfaction (Dr. P.G. Moore, pers. comm.).

An examination of the fine structure of the terminal and subterminal sensory setae groups on the first antennae of both Platorchestia platensis and Talorchestia deshayesii suggested that the first antennae may still have an important role in orientation behaviour and chemoreception (Dahl, 1973). Indeed the function and distribution of sensory setae is little different from that in fully aquatic Gammarus spp. The total number of sensory setae on various parts of the body is considerable and presumably would substantially increase the danger of desiccation, both of the general body surface and the sensory micro structures themselves, thus further restricting talitrids to moist terrestrial environments.

7.7 FEEDING

There is very little change in feeding method associated with the colonisation of land. There is a tendency towards strengthening and

simplification of the mouthparts (Hurley, 1968; Friend and Richardson, 1986), perhaps as an adaptation to chewing drier food particles. Bousfield (in Kaestner, 1970) noted that the palpi and most fine setae on the mouthparts are lost in talitrids, analagous to the trend in insects. As regards the physiology of digestion, there are few changes required to cope with land life (see chapter 2 for details). Leaf litter, which forms a large part of the diet of landhoppers (Ashton, 1975; Friend and Richardson, 1977; Richardson and Morton, 1986) can also be ingested by O. gammarellus (Perkins, 1974; see also Moore and Francis, 1985b) and O. cavimana (J.I.S. unpubl. obs.) as well as by aquatic amphipods such as Gammarus pulex (Willoughby and Earnshaw, 1982).

7.8 LOCOMOTION

Both beachfleas and sandhoppers have highly developed walking and jumping behaviour and display concomitant mophological specialisation (see chapter 2 for details). It has been suggested in this connection, that early formation and strengthening of peraeopod surfaces as found in O. gammarellus may be one of the features that have allowed the development of a land based life (Charniaux-Legrand, 1952). Certainly newly moulted O. gammarellus show great difficulty in sustaining locomotory activity (J.I.S. pers. obs.).

7.9 CUTICULAR MICROSTRUCTURES

The existence of cuticular microstructures on the outer surface of terrestrial crustaceans has been recognised for a long time, but until recently they were thought to be absent from the surface of semi- and fully terrestrial amphipods (Meyer-Rochow, 1981). If Schmalfuss (1978) is correct and such microstructures do function solely as anti-adhesive organs in terrestrial crustaceans, the lack of

such structures in the amphipods as well as the occurrence of a rougher cuticle, taken together could have been taken to indicate that terrestrial amphipods (Group IV) had colonised land in relatively recent geological time. A re-analysis of Meyer-Rochow's material, however, together with new observations on some other fully terrestrial leafhopper species has revealed the presence of complicated and highly specific patterns of cuticular microstructures (Duncan, 1985a). The presence of these microstructures is in keeping with a more ancient origin for this group (Bousfield, 1984). The evolution of such structures (possibly useful for existence in an environment characterised by sticky soil and humus particles) has not been confirmed in any beach or sandflea genus. This may be important, particularly for the beachflea species where physiological adaptation to a euterrestrial life may still be in full flux, although it should be remembered that many marine amphipods have complicated cuticular designs and structures (Cuadras, 1982).

7.10 THE PHYSIOLOGICAL ECOLOGY OF LAND INVASION BY TALITRID AMPHIPODS - AN EVOLUTIONARY SYNTHESIS

A summary of the physiological characteristics discussed earlier in this chapter together with a list of adaptations drawn from other relevant literature, is presented in Figure 7.1. They have been classified under three of the four eco-morphological headings suggested by Bousfield (1984).

The scheme is based upon the scenario envisaged by Bousfield (1984) with two main routes onto land; (i) direct, (ii) via the supralittoral zone. A recent review has confused these two options (Matsuda, 1982) but a reply by Dr. K.W. Duncan (Duncan, 1985b) has both criticised the mechanism of the evolutionary process put forward

FIG. 7.1 Physiological ecology of land invasion by talitrid amphipods

This figure opposite is a graphic depiction of suggested routes onto land taken by talitrid amphipods with indications of the physiological changes that have accompanied this transition. Modern day species are mentioned as group representatives, but it is not suggested or assumed that mechanisms present in extant species evolved directly from those of other extant species. Neither is the scheme presented necessarily as a cladistic model of talitrid evolution. It is simply a useful framework for following and predicting changes in the physiology of these animals necessitated by the move onto land. Together with the material presented in this chapter, the terrestrial adaptations as listed by Wildish, 1982a (*) are listed.

GRASSLAND (More xeric habitats)

1. Development of greater desiccation resistance.

eg. Austrotroides crenatus

1. Development of greater desiccation resistance.
2. Well developed ionic/osmotic regulation.

eg. Talorchestia patersoni

Landhopper (Gp IV,2)

1. Tendancy toward loss of bimodal breathing.
2. Unable to survive prolonged immersion.
3. Low affinity, temperature insensitive pigment?
4. Further reduction of the pleopods.
5. Better developed ionic/osmotic regulation.
6. Short amplexus time.*
7. Slow moving.*
8. Slow metabolism.*
9. Cryptozoic.*
10. Distribution: Endemic.*

eg. Orchestia sylvicola

LEAFMOULD ZONE

Landhopper (Gp IV,1)

1. Loss of bimodal respiration.
2. Unable to survive prolonged immersion
3. Low respiraory Q_{10} value.
4. Low affinity, temperature insensitive pigment?
5. Modified gills but retention of an 'aquatic' gill area.
6. Pleopods reduced or absent.
7. Low resistance to desiccation.
8. Ammoniotelic excretion.
9. Exoskeleton highly calcified.
10. Welldeveloped ionic/osmotic regulation
11. Short amplexus time.*
12. Slow moving.*
13. Cryptozoic.*
14. Slow metabolism, neotenous growth.*
15. Distribution: Endemic.*

eg. Arcitalitrus dorrieni

SUPRA-LITTORAL ZONE

Sandhopper (Gp III)

1. Retention of bimodal respiration.
2. Able to survive prolonged immersion.
3. Low respiratory Q_{10} value.
4. High affinity, temperature insensitive pigment.
5. Reduced gill area
6. Pleopods normal or reduced, never absent.
7. Low resistance to desiccation (but greater than Landhoppers IV,1).
8. Ammoniotelic excretion.
9. Good ionic/ osmotic regulation (but less so than Landhoppers IV,1).
10. Exoskeleton soft.
11. Long amplexus.*
12. Fast moving.*
13. Fast metabolism.*
14. Nocturnally active.*
15. Distribution: cosmopolitan*

eg. Orchestiagammarellus
and
Talitrus saltator

Beachflea (Gp II)

INTERTIDAL ZONE

by Matsuda (1982) and readvocated an evolutionary history for the group similar to that proposed by Bousfield (1984). While Duncan (1985b) is correct in exercising caution when relating physiological (or other) adaptations to evolutionary trends within the landhopper groups, this should not preclude periodic attempts to draw together the disparate threads of our understanding of talitrid biology. It is clear from the present assessment that, in experimental terms, very little consideration has been given to the mechanisms involved in the colonisation of land by this group. This may reflect, up until fairly recent times, adherence to an oversimplified view of talitrid phylogeny.

Despite a great deal of convergent evolution, both groups of landhoppers (IV,1 and IV,2) retain many characteristics which are highly specific and, which it is suggested, are indicative of the route they have taken onto land (Fig. 7.1). It is clear that the beachflea (eg. Orchestia, Chroestia, Transorchestia etc.) and sandhopper genera (eg. Orchestoidea, Talorchestia etc.) are highly modified for life in the supralittoral zone. Many of the features that developed in passage through the littoral zone in response to immediate habitat requirements, eg. gill reduction, increased desiccation resistance, increased suppleness of the integuement (Fig. 7.1 would not appear to be essential prerequisites for life on land. This is shown most clearly by the absence of many of these features in the Group IV:1 landhoppers even though they may be present in the Group IV:2 species which were derived from beachflea progenitors. Furthermore, this lends qualified support to the contention that, at least initially, amphipods have adapted to land by behavioural rather than physiological means (see chapter 1. Introduction). The landhoppers which have emerged via the supralittoral zone, eg.

Talorchestia patersoni, are to a certain extent, physiologically pre-adapted for more rigorous terrestrial environments (i.e. their success is due to pre-adaptations of littoral and supralittoral progenitors) than are the more ancient simplidactylate group, but continental penetration may be limited in the former not by desiccation stress but by osmo-/iono-regulatory constraints. It is suggested given the present state of our knowledge, that beachflea (Group II) and landhopper (Group IV:2) genera are physiologically hardy animals, but, that their capacity for ionic and osmotic regulation is not so well developed as is postulated for the more ancient landhopper group. It is further suggested that the degree of ionic/ osmotic regulation shown in both landhopper groups, is the key factor enabling the emergence onto land in this amphipod family.

The earliest euterrestrial landhoppers (Group IV:1), having evolved from simplidactylate palustral ancestors in high-rainfall coastal regions of Gondwanaland in the mid-Cretaceous (Bousfield, 1984), invaded land directly via the leaf litter of the newly established angiosperm rainforests. Thus these amphipods are characterised by the retention of an aquatic gill area in the absence of transitional desiccation stress (Fig. 7.1). Some of the more ancient landhoppers (Group IV:1) (and Group IV:2, as hinted at by Wildish, 1979) have also, it is suggested, developed greater powers of desiccation resistance subsequent to the invasion of land (see Friend, 1982 on Austrotroides pectinalis). The move from saturated to more xeric conditions by landhoppers (Group IV:1) may be found, in terms of physiological adaptation, to parallel the move from the littoral to the supralittoral zone by both the beachflea and sandhopper genera. This hypothesis, however, requires a great deal more work involving contemporaneous interspecific comparisons of the groups involved.

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